Smoking during pregnancy : the haematological status of smoking and non-smoking pregnant women and their offspring

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voor Luc
aan mijn ouders
en schoonouders

Abbreviations

AT-III	antithrombin III
BASO	basophilic granulocytes
DDM	D-dimers
DNA	desoxyribonucleic acid
EO	eosinophilic granulocytes
F 1+2	prothrombin fragment 1+2
FDP	fibrin degradation products
FEV1	forced expiratory volume
G	giga = 10^9
HCT	haematocrit
HFR	high fluorescence ratio
HGB	haemoglobin
IR	interquartile range
LFR	low fluorescence ratio
LYMPH	lymphocytes
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MFR	medium fluorescence ratio
MONO	monocytes
MPV	mean platelet volume
NEUT	neutrophilic granulocytes
P-LCR	platelet large cell ratio
PAI	plasminogen activator inhibitor
PAP	plasmin-α_2-antiplasmin complex
PCT	plateletcrit
PDW	platelet distribution width
PGI_2	prostacyclin I_2
PLT	total platelet count
RBC	total red blood cell count
RDW-CV	red cell distribution width coefficient of variation
RDW-SD	red cell distribution width standard deviation
RET	total reticulocyte count
RNA	ribonucleic acid
T	tera = 10^{12}
t-PA	tissue plasminogen activator
TAT	thrombin-antithrombin III complex
u-PA	urokinase plasminogen activator
WBC	total white blood cell count

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Chapter 1

Introduction

In the European Community countries 36% of the inhabitants smoke. This average is brought down by the low rates of smoking among women in southern Europe.[1] In the Netherlands 30% of all women of reproductive age smoke, compared with 36% of men. Surveys of smoking during pregnancy have shown that the prevalence of smoking in this group of women is high. Several studies have assessed that while approximately 40% of smokers may reduce their level of tobacco consumption during pregnancy[2-4], one-third of all pregnant women continue to smoke.[5,6] Nicotine dependence is the most powerful driving force for continuing the habit.[7]

Tobacco smoke and its constituents

Tobacco was introduced in Europe from South America in the 16th century. Although its potential for harm was early recognised it was taken up avidly in every society that met it. The devastating effects of smoking are caused by the many toxic substances of tobacco smoke. Tobacco smoke consists of more than 3600 different compounds[8] and its composition varies with the type of tobacco and the way it is smoked. The chief pharmacologically active ingredients are nicotine (acute effects) and tar (chronic effects). It can be divided into a particulate phase and a vapour phase. The particulate phase consists of tiny drops. Water and nicotine are its most important compounds. The vapour phase contains substances such as carbon monoxide and nitrogen oxides. Tar is defined as the condensate of smoke minus water and nicotine and contains a large variety of (aromatic) hydro-carbons.

Pharmacology

Nicotine is an alkaloid formed by the leaves of the tobacco plant (Nicotiana Tabacum). An average cigarette contains approximately 1 mg nicotine. Initially, nicotine stimulates the sympathetic and parasympathetic ganglia via a direct acetylcholinergic like action. This stimulatory phase may be brief and is followed by a prolonged ganglion blockade resulting from persistent depolarization. Similar excitatory-inhibitory phases occur in the cardiovascular and central nervous systems, as well as at the neuromuscular junction. The impact of nicotine on the central nervous system is neuroregulatory in nature, with cascading effects on physiological and biochemical functions. The dose-related effect of

nicotine on neurotransmitters and neuroendocrine responses constitute an important component of its pharmacological actions. After a cigarette has been smoked, circulating levels of noradrenaline and adrenaline increase as plasma nicotine levels rise.[9] This effect is dose-dependent. Dose-related increases were also observed in plasma levels of arginine-vasopressine, β-endorphin, adrenocorticotrophic hormone, cortisol, growth hormone and prolactine after smoking.[10] Circulating levels of thyroid releasing hormone, thyroid stimulating hormone and gonadotrophin releasing hormone were not affected by smoking.[11,12]

The nicotine metabolite cotinine is known to relax vascular smooth muscles and to dilate blood vessels in vitro.[13] It decreases blood pressure and reverses the pressor actions of nicotine in anaesthetized dogs[14] and induces electroencephalographic activation in conscious rats.[15] Cotinine is more potent than nicotine in increasing serotonin turnover in the rat cortex.[16] Little is known about the activity of cotinine concentrations found in men. Benowitz et al.[17] observed no change in blood pressure, heart rate or skin temperature after cotinine infusion, effects extremely sensitive to low concentrations of nicotine.

Thiocyanate is a metabolic byproduct of tobacco smoke. Tobacco smoke contains cyanide, most of which is rapidly converted to thiocyanate after absorption in the body.[18-19] In high doses both cyanide and thiocyanate are toxic. They can act as hypotensive agents, reduce intracellular oxygen utilization by inhibiting cytochromes, interfere with vitamin B12 metabolism, cause degenerative neurolo-gical disease and alter thyroid function.[18-21] Thiocyanate has a long half-life (14 days) but its levels may also rise moderately through exposures unrelated to smoking, such as diet (vegetables).

Carbon monoxide is one of the most important constituents of tobacco smoke. The concentration of carbon monoxide in tobacco smoke is 1-5%. It is a product of incomplete combustion of tobacco. Carbon monoxide has a high affinity for haemoglobin and thus endangers the uptake and release of oxygen. Myocardial oxygen demand is therefore increased.[22,23] High concentrations of carboxyhaemoglobin (COHb) in the blood (15% in heavy smokers) can lead to changes in behaviour and can have negative effects on the vessel walls.[22]

Various biochemical procedures have been used to estimate exposure to tobacco smoke: concentrations of nicotine, cotinine, carbon monoxide, carboxyhaemoglobin and thiocyanate in blood have been determined.

Nicotine is pharmacologically the most active constituent of tobacco smoke. Fifty to ninety percent of nicotine in smoke is absorbed by the smokers and can be detected in the blood shortly after smoking.[24] In adults the elimination half-life of nicotine in blood is approximately 120 minutes and 60% of the substance is metabolized to cotinine.[17,25] This is mainly accomplished by oxidation of nicotine in the liver.[24] About 9% of the nicotine uptake is excreted unchanged in the urine; 60% is converted into cotinine of which 10% is excreted as such in the urine.[25] The level of cotinine in the blood depends on the rate of generation from nicotine and the rate of elimination from the body.[26]

Cotinine is slowly cleared from the body (total body clearance averaging 72 ml/min) and is primarily eliminated by the liver.[26] Renal clearance accounts for about 17% of total clearance. In contrast to nicotine clearance, cotinine clearance is insensitive to changes in urinary pH. Because of the low rate of metabolism and renal excretion, the half-life ($t_{1/2}$) of cotinine is approximately 15 hours (10-20 h).[24] As a consequence of the long cotinine $t_{1/2}$ there is relatively little fluctuation in blood concentrations throughout the day. Cotinine concentrations rise gradually in the course of a smoking day, reach a maximum at the end of the day, and persist in relatively high concentrations overnight.[26] The plasma nicotine and cotinine levels directly after smoking a cigarette depend more on the way the cigarette is smoked than on its nicotine yield.[27] Smokers of lower yield nicotine cigarettes have a tendency to compensate by increasing smoke inhalation.

Various investigators have chosen thiocyanate as a marker of cigarette smoke exposure as it has been reported to be an effective biochemical marker of exposure[16,20,21] and it has a long half-life (14 days) compared to the half-life of nicotine (< 120 min) and carboxyhaemoglobin (4 hours).[19,27-29] Carbon monoxide and carboxyhaemoglobin have also been used as indicators of cigarette consumption.[30-33]

The five mentioned methods for the determination of cigarette exposure differ widely in availability and costs. Measurements based on nicotine have the advantage of being specific to tobacco but acquire expensive laboratory instrumentation. Levels of thiocyanate, carbon monoxide and carboxyhaemoglobin are easier to determine but may be artificially raised through exposures unrelated to smoking, such as traffic exhaust gases (CO) and diet (thiocyanate).[34] A few studies have attempted to compare the various biochemical tests.[31-35] Hill et al.[35] conclude that plasma and urinary nicotine and cotinine are valid indicators of smoke absorption, while carboxyhaemoglobin levels correlate well with cigarette smoke inhalation. Pojer et al.[33] reach the same conclusion. Jarvis et al.[34] are of the opinion that measurements of cotinine are best used in discriminating smokers from non-smokers and are the most suitable tests for research protocols when accurate categorization is essential.

To understand the effects of maternal smoking on the fetus and neonate an estimate of the degree of fetal exposure to the constituents of cigarette smoke should be included. The amount of products of tobacco smoke transmitted to the fetus varies consistently, depending on the proportion of each cigarette consumed, frequency of puffing, depth of inhalation and maternal metabolism.[34,35] After birth, the metabolism of the neonate affects the levels and duration of exposure to smoke products. For this reason, biochemical measurements of tobacco products such as maternal and cord blood levels of thiocyanate were carried out by Bottoms et al.[36] They studied fetal serum thiocyanate levels in relation to maternal passive smoking. Among non-smokers, fetal thiocyanate levels were increased in association with passive smoking ($p < 0.05$). During pregnancy nicotine and cotinine pass the placenta and expose the fetus to concentrations similar to those in the blood of the smoking mothers.[37,38] After birth the child may still be

exposed to these substances through passive smoking or breast feeding.[37,39,40] Etzel and co-workers[38] performed cotinine measurements in urine of neonates of smoking and non-smoking women. Cotinine was measured by radioimmunoassay. The urine concentrations found in neonates of smoking mothers were generally lower than in their mothers, but higher than those in neonates of non-smokers.[41,42] Klein et al.[43] measured the nicotine and cotinine content of maternal and neonatal hair by radioimmunoassay. A positive correlation between maternal exposure to nicotine and cotinine and the accumulation of these compounds in neonatal hair was found. The authors suggest that measurements of hair may provide a better estimate of long-term systemic exposure to the toxic constituents of cigarettes and may thereby yield a better prediction of fetal risk.

Smoking and blood cells

Various reports have established that total white blood cell counts are significantly higher in smokers compared to non-smokers.[44-46] This rise has been attributed mainly to monocyte release.[47-49] An increased white blood cell count, found in chronic smokers, has been associated with a higher risk of mortality from cancer.[50] Several investigators have addressed the question of whether smoking can change the functional characteristics of inflammatory cells. They have produced evidence that inflammatory cell migration can either be inhibited or increased by smoking.[51-54] Bridges et al.[52] found that aldehydes, also being components of tobacco smoke, are potent inhibitors of neutrophil chemotaxis. However, Totti et al.[53] showed that nicotine may be chemotactic for neutrophils and may enhance neutrophil responsiveness to chemotactic peptides. Other investigators did not observe this finding.[54] Considering the chemical complexity of smoke and the individual variations in smoking patterns, it remains difficult to resolve whether smoking increases or decreases inflammatory cell responsiveness.

Carbon monoxide, the main component of the vapour phase of tobacco smoke, binds to haemoglobin, replaces oxygen and thus produces hypoxemia[55], which in the long term can cause polycythemia in heavy smokers.[56] Considerable evidence has accumulated to link smoking and polycythemia. In an American study, haematocrits of blood donors who smoked were significantly higher than those of non-smoking donors.[57] A Danish survey showed a statistically significant correlation between cigarette consumption and the haematocrit.[58] A Canadian group described elevations of the haematocrit, haemoglobin and red blood cell count in smoking subjects, with a decline of these variables when the subjects refrained from smoking.[59] The British[47] studied the haematocrit and mean corpuscular volume in smokers, ex-smokers and those who never smoked. Those who had never smoked and the ex-smokers had similar haematocrit values. The haematocrit was much higher in current smokers and there was a strong dose-response relationship. The mean corpuscular volume was also highest in current

smokers and it showed a significant dose-response correlation with the amount of cigarettes smoked.

In view of a possible association between smoking, an altered state of the blood platelets and the development of coronary atherosclerosis, several investigators have focused on the influence of smoking upon platelets.[60-62] Cigarette smoke has been reported to induce platelet activation, an effect mediated mainly by nicotine, and to increase the platelet adhaesion to the vessel wall.[63-66] Aggregation of platelets is acutely increased by cigarette smoke.[67-69] Tobacco smoke reduces the production of endothelial cell prostacyclin, an inhibitor of platelet aggregation.[70] Smoking also causes acute and chronic inhibition of platelet cyclo-oxygenase, which increases the biosynthesis of thromboxane.[71] Thromboxane is a potent vasoconstrictor and platelet agonist thus leading to stimulation of platelet aggregation. A further finding is that platelet survival is shortened in smokers. This is considered to be an indirect indicator of platelet activation.[70]

Maternal smoking during pregnancy may create a condition of chronic hypoxia for the fetus. This can be the result of the replacement of oxyhaemoglobin by carboxyhaemoglobin.[21] Additionally, structural changes in the placenta[72] as well as decreased placental blood flow[73] may also impair the oxygen supply to the fetus. Increased haemoglobin and haematocrit levels have been reported in infants of smoking mothers.[74] This fits the hypothesis that maternal smoking creates a hypoxic condition for the fetus, stimulating the erythropoiesis. Meberg et al.[75] found that the haematocrit levels of neonates were higher with increasing cigarette consumption by the mother. This indicates that the higher the maternal smoking level, the stronger the hypoxic stimulus for fetal erythropoiesis. In association with intrauterine growth retardation a transitory postnatal thrombocytopenia was more frequently found among infants of smoking mothers than among those of non-smokers.[76] No data are available on leucocyte counts in neonates of smoking mothers.

Smoking and haemostasis

Several lines of evidence suggest that smoking affects the coagulation system as shown by higher plasma fibrinogen and thrombin-antithrombin III (TAT-III) levels.[77] Studies of fibrinolysis in smokers have shown variable results.[78-80] Two studies reported that fibrinogen levels were higher and that fibrinolysis was decreased in subjects who smoke.[78,81] In two other studies[82,83], fibrinolytic activity was reported to be enhanced after smoking, whereas an other group of investigators found no influence of smoking on fibrinolysis.[84] Kimura et al.[85] studied the acute effect of cigarette smoking on haemostasis and found simultaneous increases in both coagulability and fibrinolysis during smoking.

Smoking and pregnancy

Cigarette smoking is associated with a dose-related reduction in fecundity and fertility.[86] Once smoking women get pregnant they have an increased risk of having a spontaneous abortion.[87] An increased risk of antepartum haemorrhage has been described.[88] Tobacco use during pregnancy is associated with an increase in low birth weight due to both preterm delivery (nicotine has an oxytocin-like effect upon uterine contractility[89]) and the delivery of small for gestational age infants.[90,91] An average decrease in birth weight of 200 g has been reported.[90] The association between smoking and perinatal death is disputable. In a review article, McIntosh stated that only five out of seventeen studies reported a significant increase of stillbirth among smokers. An increased risk of early death was reported in four studies. Some tobacco-related deaths are probably due to the increased risk of malformations.[92] The deleterious effects of smoking might be mediated by direct placental DNA damage.[93] Children of mothers who smoke are admitted twice as often to the hospital for pulmonary problems.[94] Studies on maternal smoking and childhood cancer have proved inconclusive.[95-97]

Objectives of the study

The mechanisms by which cigarette smoking has a negative effect on pregnancy and pregnancy outcome are complex and multifactorial. The aim of this study was to investigate whether these effects are mediated or reflected by changes in haematological variables in smoking pregnant women and/or their offspring. Consequently, the following objectives were pursued:

1. The effect of smoking on blood cells and cell indices in mothers (chapter 4, 5, 6, and 7) and their newborns (chapter 9).
2. The sex-related differences of nicotine exposure in neonates (chapter 10).
3. The simultaneous effects of smoking and pregnancy on haemostasis (chapter 8).
4. The haemostasis in neonates of smoking and non-smoking mothers (chapter 11).
5. To estimate the degree of neonatal exposure to the constituents of cigarette smoke. For this purpose cotinine measurements were carried out in maternal blood plasma and cord blood plasma (chapter 3).

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Chapter 2

Smoking and reproduction

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The literature has been updated

In the Netherlands approximately 30% of all women of reproductive age smoke, compared with 36% of men.
Cigarette smoking is associated in women with a dose-related reduction in fecundity and fertility and in men with a reduction in semen quality. Smoking has a negative effect on pregnancy: increased rates of antepartum bleeding and placenta praevia have been described. Smoking is also associated with increases in the rates of spontaneous abortion, low birth weight and perinatal death. Some tobacco-related perinatal deaths are due to an increased risk of serious malformation. Children of mothers who smoke are admitted twice as often to the hospital for pulmonary problems. However, studies on maternal smoking and childhood cancer have proved inconclusive.
As smoking is a major modifiable risk factor in pregnancy, counselling of women who smoke should have a high priority in antenatal care. The purpose of this review is to summarize the influence of cigarette smoking on the reproductive process.

Smoking and fertility

Female fertility

Baird et al[1] found that 28% of women who smoked conceived in the first cycle after stopping contraception compared with 38% of non-smokers and that smokers were 3.4 times more likely to take more than a year to conceive. In a study on 35,973 women an increased infertility rate (35%) was observed among smokers.[2] Daling et al[3] found an increased risk of primary tubal infertility among smokers. In a British study, reduced fertility among women smokers was strongly correlated with the number of cigarettes smoked per day and women smoking more than 15 cigarettes per day had a relative fertility of 0.79. The fertility of women who had stopped smoking was the same as that of the non-smokers.[4]
The muscular tone of the oviduct and the amplitude of its contractions are increased in smokers.[5] Smoking therefore seems to affect tubal function and tubal transport of the embryo. These observations suggest a mechanism for the increased risk of ectopic pregnancy in smokers. Chow et al[6] found that the relative risk of ectopic pregnancy was increased in both former smokers (relative risk 1.6) and current smokers (relative risk 2.2). The suggestion that cigarette smoking is a risk factor for ectopic pregnancy lends support for a possible relationship between cigarette smoking and pelvic inflammatory disease. Marchbanks et al[7] investigated this hypothesis and found that both current and former cigarette smoking were associated with a statistically significant doubling of the relative risk of pelvic inflammatory disease, although no dose-response relationship was observed. The mechanism by which cigarette smoking could yield an increased relative risk of pelvic inflammatory disease is still unclear.

Nicotine has a negative effect on the viability and maturity of oocytes.[8] Rosevear et al[9] studied the relation between cotinine concentrations in preovulatory ovarian follicles and the fertilizability of the oocyte during in vitro fertilization. They showed that cigarette smoking, as indicated by the presence of cotinine in preovulatory follicular fluid, was associated with reduced fertilization of eggs to approximately two-thirds of the normal rate.

The levels of luteinizing hormone are reduced in women who smoke, the LH surge is blocked and the level of prolactin is increased.[10] The aromatase activity in the ovaries is lower while 2-hydroxylation of oestradiol in the liver is activated, leading to lower levels of oestrogen[11] and higher levels of androstenedione.[12] Consequently, fertility may be impaired by irregular ovulation and secondary amenorrhoea. A higher thiocyanide concentration in cervical secretion is said to have a negative influence on sperm motility.[13]

Male fertility

During recent years the influence of environmental factors on sperm quality has become an important topic. The results of studies that have been performed to analyze the relationship between cigarette smoking and semen quality have been contradictory. Some investigators have reported a negative effect of smoking[14-18], while others have failed to demonstrate any adverse effect.[19-23] Shaarawy et al[17] studied the serum endocrine profile and semen characteristics of male smokers and found that smoking led to increased oestrogen and decreased testosterone concentrations. Prolactin and LH levels rose and FSH fell, resulting in decreased sperm production. Vasopressin levels also increased while those of testosterone fell. High vasopressin levels have been shown to relate to low sperm count and motility.[24] Osser et al[25] studied the semen of 186 male smokers and 164 non-smokers in connection with investigation of their infertility. No statistically significant effect of cigarette smoking on sperm was detected. Marshburn and co-workers[26] found that smoking alone did not greatly affect spermatozoa, but that the combination of smoking more than 20 cigarettes and drinking more than four cups of coffee per day significantly reduced sperm motility. The combination of smoking and varicocele has also been found to be unfavourable.[27]

Smoking and pregnancy

Surveys of smoking in pregnancy have shown that the prevalence of smoking is high and approximately one third of all pregnant Western women continue to smoke. Many factors have been associated with smoking in pregnancy including poor education, lower social class, having a partner who smokes, being single, and being under 20 years of age.[28] The main reasons given by pregnant women for smoking include those related

to mood control (e.g. to calm down, enjoyment, out of boredom) and addiction.[29] Nicotine dependence is the most powerful driving force for continuing the habit.
Nicotine is an alkaloid which is the primary component of the particulate phase of tobacco.[30] Nicotine exerts a constricting effect on uterine blood vessels and interferes with the blood supply to the fetus. It also has an oxytocin-like effect upon uterine contractility[31], a direct vasoconstrictor effect on the fetus[32], and produces capillary damage in the placenta.[33] The teratogenic effects of smoking may be caused predominantly by nicotine itself and by its metabolite cotinine, both of which pass easily through the placental barrier to the fetal central nervous system[33]. Biological markers show that the fetus is heavily exposed to several components of tobacco smoke.[34]
Carbon monoxide is the primary component of the vapour phase of tobacco smoke.[30] It binds to haemoglobin and produces a conformational change in oxyhaemoglobin. This results in a shift of the oxygen dissociation curve leading to diminished transmission of oxygen to the tissues at a given oxygen tension and thus causing a mild form of hypoxia.[35]

Maternal lung function

Lung function changes during pregnancy. Although maximum flow rate and one-second forced expiratory volume (FEV_1) are not measurably altered[36], the reduced total pulmonary resistance and increased airway conductance[37] suggest a bronchodilator effect. This has been attributed to an increased concentration of progesterone, which relaxes smooth muscle.[38] Das et al[39] investigated the effect of cigarette smoking on maternal airway function during pregnancy. All spirometric parameters were significantly lower in smokers than in non-smokers. Forced vital capacity, FEV_1 and the ratio of these two parameters were minimally reduced. The progression of small airway disease was related to the level of cigarette exposure. Greater reductions in forced expiratory flow rates and instantaneous flows in smokers suggest an increase in small airway resistance and early small airway disease. The changes in airway function in pregnant smokers were similar to those observed in non-pregnant smokers. The authors concluded that the bronchodilator effect expected in pregnancy was not sufficient to overcome the deleterious effect of cigarette smoking.

Antenatal complications

Placental morphology

Smoking during the first months of pregnancy induces morphologic changes of the placenta. Jauniaux et al[40] found an increased thickness of the villous membrane and the trophoblastic layer in the placentas of heavy smokers.

Demir et al[41] studied human placental villi from smoking and non-smoking pregnant women in all three trimesters of pregnancy. The mean birth weight and placental weight in smokers were decreased depending on the cigarettes smoked per day in the third trimester. The villi from smokers had abnormalities of the microvilli, focal syncytial necrosis, decreased syncytial pinocytotic activity, and degenerated cytoplasmic organelles. The thickness of the trophoblastic layer increased and fetal capillaries were damaged.

Antepartum haemorrhage

Underwood et al[42] found an increased rate of antepartum haemorrhage in smokers compared with non-smokers. Four studies have demonstrated a positive correlation between placenta praevia and maternal cigarette smoking. Kramer et al[43] reported an increased risk of placenta praevia in smokers (odds ratio 1.9: 95% confidence interval, 1.5 to 2.8). Meyer et al[44] observed 25% and 92% increase in the prevalence of placenta praevia in pregnant women smoking less than, or more than, one pack of cigarettes per day respectively. Williams et al[45] conducted a case-control analysis of 69 women with placenta praevia and 12,351 controls. The results suggested that smoking during pregnancy was a determinant of placenta praevia. However, they did not confirm Naeye's findings[46] that the frequency of placenta praevia was more strongly associated with the number of years that mothers had smoked than with their smoking habits during pregnancy. The reason for the increased rate of placenta praevia may relate to the fact that placental weight is 0.7-3.6% greater in smokers.[47-49] In one of these studies it was found that increased placental weight was accompagnied by 1.9% - 2.4% decrease in placental thickness and by a 0.4% - 1.3% increase in the smallest diameter of the placentae of smokers compared with those of non-smokers.[49] Williams et al[45] suggested that carbon monoxide-induced hypoxaemia might result in compensatory placental hypertrophy. Placentae with increased surface areas are more likely to cover the cervical os.

Pre-eclampsia

The incidence of pre-eclampsia and eclampsia seems to show an inverse dose-response relationship with cigarette consumption. Kullander and Kallen[50] reported pre-eclampsia in 11% smokers compared with 16% of non-smokers in a Swedish study of more than 6000 pregnant women. This association has been ascribed to the hypotensive effects of thiocyanide derived from cyanide present in cigarette smoke and found in the blood of smokers.[51]

Pregnancy outcome

Abortion

Several studies have suggested an increase in the spontaneous abortion rate in smokers compared with non-smokers. As early as 1931, Mgalobeli[52] reported that women who worked in tobacco factories had fewer pregnancies, more abortions and a higher infant mortality rate than those who did not. Spontaneously aborted fetuses of women who smoke have a lower frequency of congenital abnormalities.[53] It has been suggested that smoking can interfere with implantation. However in a study from Finland, Hemminiki et al[54] were unable to find a significant effect of smoking on spontaneous abortion. Sandahl[55] also showed no effect of smoking on abortion risk. The results of this study actually suggested a slightly reduced odds ratio for spontaneous abortion in smokers.

Birth weight

Between 1957 and 1990 over a hundred publications, based on studies of more than half a million births, reported that women who smoked during pregnancy had infants of lower birth weight than women who did not.[56] Tobacco use during pregnancy is associated with an increase in low birth weight due to both preterm birth and the delivery of small for gestational age infants.[57,58] Maternal tobacco use has been reported to cause an average decrease in birth weight of 200 g[59]. Peacock et al[60] related birth weight to the number of cigarettes smoked per day and found that there was a reduction in weight of 0.19 % per cigarette. There was an 8% reduction in birth weight in the children of mothers who smoked more than 13 cigarettes per day compared with those who smoked less than this.

The strength of the association between maternal smoking and lower birth weight, the consistency of the findings and the existence of dosage effects, all add up to strong evidence that the association is causal.[56]

Perinatal mortality

The association between smoking and perinatal death was initially disputed.[61] In a review article, McIntosh stated that only five of seventeen studies reported a significantly increased risk of stillbirth among smokers.[62] An increased risk of early death among the infants of smokers was reported in four studies, but none reached statistical significance. Cnattingius et al[63] studied risk factors for late fetal and early neonatal death. The overall rates were found to be 3.5 and 3.1 per 1000 respecti-vely. The relative risk for early neonatal mortality was increased for multiple birth (4.9) and smoking (1.2). Smokers aged under 35 had a relative risk of late fetal death ranging from 1.1 to 1.6, while the risk was doubled if mothers were above this age. Kleinman et al[64] found that

primigravidae who smoked less than one packet of cigarettes per day had a 25% greater risk of mortality compared with non-smokers while those who smoked one or more packets had a 56% greater risk. The prevalence of smoking in this population was 30%. It was estimated that if all pregnant women stopped smoking, the number of fetal and infant deaths would be reduced by approximately 10%.

Congenital malformations

Some tobacco-related prenatal deaths are probably due to the increased risk of serious malformation.[65] An American epidemiological study showed a 1.6-fold increase in the risk of malformation in mothers who smoked more than 20 cigarettes per day during pregnancy.[66] Swedish workers reported an increased risk of cleft lip and palate in children of mothers who smoked.[67] Rivrud et al[68] found an increased risk of cleft palate and malformations of the central nervous system. This risk was dose-dependent. Czeizel and co-workers[69] found that smoking during pregnancy raises the relative odds for terminal transverse limb deficiencies. These malformations may be caused by mutagens, some of which have been demonstrated in amniotic fluid. Recently a study of cytotrophoblast cells from pregnant women showed placental deoxyribonucleic acid (DNA) damage among smokers. These findings suggest that the deleterious effect of smoking could be mediated by direct placental DNA damage.[70]

Paediatric outcome

Lung function

Several studies have reported an association between smoking habits of parents and pulmonary morbidity in their children.[71-73] Harlap and Davies[72] found that the prevalence of hospital admission for bronchitis or pneumonia was twice as high in infants of smokers compared with infants of non-smokers. Tager et al[73], using pulmonary function studies, documented decreased lung growth during childhood and adolescence in association with parental smoking. The effect on respiratory morbidity appears to be more, although not exclusively, related to maternal rather than paternal smoking. Most studies have also found a greater effect of maternal smoking on the pulmonary function of female children. Whether this is caused by exposure before or after birth is uncertain. It may relate to the fact that mothers spend more time with their children and that girls share more activities with their mothers than boys. Another possibility might be that maternal smoking during pregnancy retards fetal lung development. Bassi et al[74] developed a rat model of maternal smoking and demonstrated that fetuses suffered from growth retardation with a predominant effect on lung growth. Deficient formation of pulmonary septa suggests that growth of the connective tissue of the fetal lung may have

been impaired during pregnancy. Septal growth may have been reduced by a deficiency of elastic tissue. It is not known whether the lung can recover from structural alterations due to antenatal maternal smoking. A study by Taylor and Wadsworth[75] supports the concept that maternal smoking influences the incidence of respiratory illness in children mainly through a congenital effect, and only to a lesser extent through passive exposure after birth. Using the British Births Survey, they first confirmed that maternal, but not paternal, smoking was significantly associated with the incidence of bronchitis and hospital admission for lower respiratory tract illness in early life. They were then able to identify subsets of women who either smoked during pregnancy but not after delivery or who smoked only after their children were born. Reported rates of admission to hospital for lower respiratory tract diseases were found to be as high in children born to mothers who stopped smoking during pregnancy as in those whose mothers smoked during and after pregnancy. No differences in the rates of hospital admission were found in children whose mothers started to smoke only after delivery as compared with children of mothers who had never smoked.

Neuropsychological problems

Children exposed to maternal smoking in utero are said to have significantly more neuropsychological deficits than children who have not been exposed. Studies of cognitive function of 3-year old children born to mothers who smoked more than 10 cigarettes per day showed a statistically lower level of performance as compared to children of mothers who stopped smoking during pregnancy.[76] These differences in cognitive functioning persisted after excluding the confounding effects of environmental factors, characteristics of the child and gestational age at birth. However Tong et al[77], in a review article on maternal smoking and neuropsychological childhood development, concluded that the evidence for maternal smoking during pregnancy being a cause of neuropsychological deficits was inconclusive. The effects might have been due to uncontrolled or unknown social and environmental factors.

Childhood cancer

Transplacental carcinogenesis has been demonstrated in animal experiments with a number of compounds present in tobacco smoke, including several nitrosamines and polyaromatic hydrocarbons.[78] Cigarette smoke condensate can also increase the number of tumours and hyperplastic laesions in the offspring of treated animals.[79]
The available evidence regarding cancer risks in children of mothers who smoke during pregnancy is inconclusive. Only the studies by Stjernfeldt et al[80] and John et al[81] suggested a clear dose-related increase in cancer risk, which was particularly marked for acute leukaemias. Pershagen et al[82] performed a cohort study, using information from the National Swedish Medical Birth and Cancer Registraties. The maximum follow up

age was 5 years. They found no increase in overall cancer risk in children of mothers who reported smoking during pregnancy. Further studies are needed to determine if smoking in pregnancy induces cancer in childhood or later life.

Sudden infant death syndrome

Sudden infant death syndrome (SIDS) is a major cause of post-neonatal mortality in industrialized countries. In addition to prone sleeping position, exposure to tobacco smoke is one of the most important epidemiological risk factors associated with SIDS.[83-85] Maternal smoking during pregnancy and nursing increases the risk of SIDS in the newborn in proportion to tobacco consumption: moderate smoking by a factor five.[86] Why tobacco exposure increases the risk of SIDS is unknown. What is known is that parental smoking is associated with lower respiratory tract infections[87] and that nicotine exposure of the fetus and neonate induces alterations in neurotransmission in the central nervous system[88] and affects cardiovascular control.[89] Nicotine exposure of rat fetuses and pups has been shown to impair the ability to increase heart rate in response to adrenergic stimulation.[89] An increased predisposition for cardiac arrhythmias and a reduced heart rate variability have been demonstrated in a number of infants who subsequently died of SIDS.[90] Milerad et al[91] found high nicotine and cotinine levels in pericardial fluid in victims of SIDS.

Conclusion

The negative influence of cigarette smoke and its components on reproduction has been reviewed. Smoking is a major modifiable risk factor in pregnancy. Randomized trials of the effects of counselling in pregnancy have documented an increased rate of discontinuing smoking.[92,93] Counselling of women who smoke should have a high priority in antenatal care, not only to prevent low birth weight but also to reduce exposure of infants to environmental smoke. Pregnant women who continue to smoke should be counselled to stop for their own health and the health of their unborn child.

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Chapter 3

Cotinine concentrations in plasma of smoking pregnant women and their infants

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Abstract

In the Netherlands 30% of all women of reproductive age are habitual smokers. One third of these women continue to smoke during pregnancy. Tobacco smoke consists of more than 3600 different compounds. One of its chief pharmacologically active ingredients is nicotine of which 60% is metabolized to cotinine. Cotinine is the best available biochemical marker of nicotine consumption because it is specific for tobacco smoke exposure and it has a relatively long mean $t^1/_2$ of 15 hours.

In the present study nicotine and cotinine concentrations were measured in 25 smoking and 25 non-smoking healthy pregnant women. In all 25 non-smoking pregnant women nicotine and cotinine levels were < 10 µg/ml. Light smokers (< 10 cigarettes/day) were found to have nicotine blood levels < 10 µg/ml and cotinine levels varying between 40 and 99 µg/ml. Heavy smokers (≥10 cigarettes/ day) had nicotine levels < 10 µg/ml, but high cotinine levels varying from 115 to 199 µg/ml.

Cotinine measurements were also determined in 25 neonates of non-smoking mothers and in 34 neonates of smoking mothers. In 9 of these 34 newborns the relationship between maternal and neonatal cotinine concentrations was investigated. Cotinine levels in neonates of non-smokers and women who smoked less than 10 cigarettes/day were below the detection limit of 10 µg/ml. Cotinine values in neonates whose mothers smoked ≥ 10 cigarettes/day were significantly higher than in those whose mothers smoked ≤ 10 cigarettes/day, but significantly lower than in their mothers.

The results of this study confirm that cotinine is more useful than nicotine in discriminating non-smokers, light and heavy smokers. Cotinine concentrations were significantly lower in the neonates than in their mothers, but there was a strong positive linear relationship between maternal and neonatal cotinine concentrations.

In the Netherlands 30% of all women of reproductive age are habitual smokers. Surveys of smoking during pregnancy have shown that the prevalence of smoking in this group of women is high. Several studies have assessed that while approximately 40% of smokers may reduce their level of tobacco consumption during pregnancy, one third of all pregnant women continue to smoke.[2,3] Nicotine dependence is the most powerful driving force for continuing the habit.[4]

Tobacco smoke consists of more than 3600 different compounds[5] and its composition varies with the type of tobacco and the way it is smoked. The chief pharmacologically active ingredients are nicotine and tar. An average cigarette contains approximately 1 mg nicotine.

Cigarette smoking during pregnancy is associated with a well-documented increase in perinatal mortality and morbidity rates.[6,7] Most of the adverse effects of smoking are related to chronic fetal hypoxia arising from decreased uteroplacental perfusion and increased levels of carboxyhaemoglobin in fetal blood.[8] These effects are mainly caused

by nicotine, tar and carbon monoxide. Nicotine also exerts significant haemodynamic effects in the mother by increasing arterial blood pressure and heart rate.[9]
Fifty to ninety percent of nicotine in smoke is absorbed by the smokers and can be detected in the blood shortly after smoking.[10] In adults the elimination half-life of nicotine in blood is approximately 120 minutes and 60% of the substance is metabolized to cotinine[11,12], of which 10% is excreted as such in the urine.[12] Cotinine is slowly cleared from the body and is primarily eliminated by the liver.[13] Renal clearance accounts for about 17% of total clearance. Because of the low rate of metabolism and renal excretion, the half-life ($t^1/_2$) of cotinine is approximately 15 hours (10-20 h).[10] As a consequence of the long $t^1/_2$ there is relatively little fluctuation in blood concentrations throughout the day. Various biochemical procedures have been used to estimate exposure to tobacco smoke: blood concentrations of nicotine, cotinine, carbon monoxide (CO) carboxyhaemoglobin (COHb) and thiocyanate have been determined. Levels of thiocyanate, CO and COHb are easy to determine but may be artificially raised through exposures unrelated to smoking, such as traffic exhaust gases (CO) and diet (thiocyanate).[14]
A few studies have attempted to compare the various biochemical tests. Hill et al[15] concluded that plasma and serum cotinine are valid indicators of smoke absorption, while COHb levels correlate well with cigarette smoke inhalation. Pojer et al[16] reached the same conclusion. Jarvis et al[14] are of the opinion that measurements of cotinine are best used in discriminating smokers from non-smokers.
While the effects of nicotine on the pregnant mother and her fetus have already been discussed, little is known about the effects of cotinine. Benowitz et al[11] observed no change in blood pressure, heart rate or skin temperature, effects extremely sensitive to low concentrations of nicotine. Keenan et al[17] assessed that cotinine has psychoactive properties. Clark et al[18] found that the pharmacodynamic activity of cotinine is only 1/100 of that of nicotine.
Even less is known about the effect of smoking on the actual concentrations of cotinine in smoking pregnant women and their newborns. For this purpose cotinine levels were measured simultaneously in maternal blood plasma (study group 1) and cord blood plasma (study group 2).

Subjects and methods

Nicotine and cotinine concentrations were measured in 25 smoking and 25 non-smoking healthy pregnant women. The measurements were done in the second trimester of their pregnancy. This checkpoint was chosen because Sexton et al[19] indicated that by the time a smoker reaches her second trimester of pregnancy she will either quit smoking or will continue to smoke until delivery. A second study group consisted of 25 neonates of non-smoking mothers and 34 neonates of smoking mothers. Of 9 of these 34

Table 1. Nicotine and cotinine values in light and heavy smokers

	Light smokers (< 10 cigarettes/day) (n=13)		Heavy smokers (≥ 10 cigarettes/day) (n=12)	
Reference range	Nicotine < 10 µg/ml	Cotinine 10-100 µg/ml	Nicotine > 10 µg/ml	Cotinine ≥ 100 µg/ml
	< 10	85	< 10	119
	< 10	99	< 10	115
	< 10	63	< 10	126
	< 10	84	< 10	152
	< 10	66	< 10	124
	< 10	67	< 10	127
	< 10	67	< 10	135
	< 10	40	< 10	184
	< 10	85	< 10	150
	< 10	42	< 10	198
	< 10	64	< 10	199
	< 10	87	< 10	132
	< 10	81		

Table 2. Cotinine concentrations in smoking mothers and their newborns

Cigarettes/day	Neonate Cotinine µg/ml	Mother Cotinine µg/ml
5	< 10	48
5	< 10	< 10
6-7	< 10	65
10	< 10	55
10	< 10	42
10-15	67	68
10-15	78	82
17-20	102	171
> 20	110	173

newborns the mothers were investigated to study the relationship between maternal and neonatal cotinine concentrations. In this second group only cotinine levels were determined. Nicotine as well as cotinine levels were determined by gas chromatography.[20] The measurements were carried out by the Department of Toxicology, University Hospital, Maastricht, the Netherlands. A Hewlett Packard 5890A gaschromatograph

was used. The detection limit for nicotine and cotinine was 10 µg/ml. Reference values for light (< 10 cigarettes/day) and heavy smokers (≥10 cigarettes/day) are mentioned in Table 1. The nicotine and cotinine measurements of the mothers were performed in venous blood plasma. Blood samples were drawn between 8.30 and 9.00 a.m., at least one hour after the last cigarette was smoked. The cotinine measurements in the neonates were performed in venous cord plasma. Venous blood samples of the newborns were obtained from the clamped umbilical cord immediately after delivery. Venous blood samples of their mothers were drawn during labour, 2-5 hours after the last cigarette was smoked. The Mann-Whitney-U test was used to compare the differences between the cotinine concentrations in neonates of smoking and non-smoking mothers. The Wilcoxon signed rank test was used to test the significance of differences in the plasma cotinine concentration between smoking mothers and their newborns. The Spearman rank correlation coefficient was used to estimate the correlation between maternal and neonatal cotinine concentrations.

Results

In all 25 non-smoking pregnant women nicotine and cotinine levels were < 10 µg/ml. The smoking group consisted of 13 women who reported smoking 5-10 cigarettes/day (light smokers) and 12 women who reported smoking 10 or more cigarettes/day. The light smokers were found to have nicotine blood levels < 10 µg/ml and cotinine levels varying between 40 and 99 µg/ml. Heavy smokers had nicotine levels < 10 µg/ml, but high cotinine levels varying from 115 to 199 µg/ml. Of the women who reported smoking more than 15 cigarettes/day (heavy smokers), 3 had cotinine values > 175 µg/ml.

Cotinine levels in neonates of non-smokers and women who smoked less than 10 cigarettes/day were below the detection limit of 10 µg/ml. In Fig 1 the cotinine concentrations in neonates are displayed in relation with their mothers' smoking habits. Cotinine values in neonates whose mothers smoked ≥ 10 cigarettes/day were significantly higher ($p < 0.001$) than in those whose mothers smoked < 10 cigarettes/day, but significantly lower than in their mothers ($p < 0.01$). There seems to be a threshold of around 10 cigarettes/day. The cotinine concentrations in the 9 smoking mothers and their infants are shown in Table 2. The correlation between cotinine concentrations in maternal and umbilical vein plasma is illustrated in Fig 2. The Spearman rank correlation coefficient was 0.912 with a p-value of 0.001.

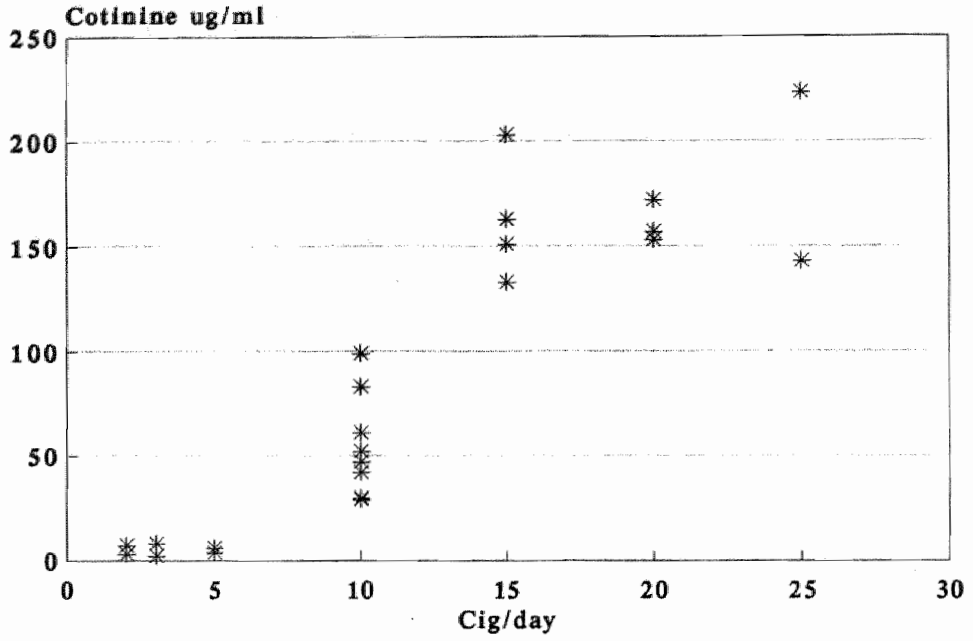

Fig 1. Cotinine concentrations in umbilical vein plasma in neonates of smoking mothers

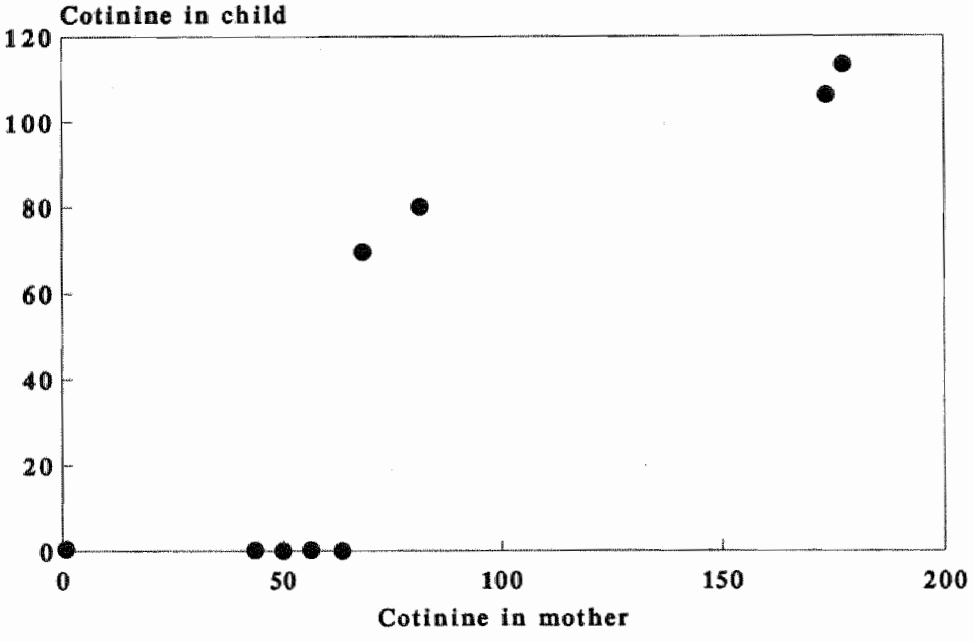

Fig 2. Correlation between cotinine concentrations (µg/ml) in umbilical vein and maternal vein plasma (rs= 0.912 p< 0.001)

Discussion

Cotinine is a principal metabolite of nicotine and has a $t^1/_2$ of about 20 h and is the best available biochemical marker of nicotine consumption.[21-24]. The cotinine measurements in our first study group confirm that cotinine measurements are more useful in discriminating non-smokers, light and heavy smokers than nicotine measurements. This is in accordance with earlier findings.[23-25] Nicotine levels in smokers were below the detection limit of 10 µg/ml because blood samples were taken at least one hour after the last cigarette was smoked. In adults the elimination half-life of nicotine in blood is approximately 120 minutes. It is of importance for other studies to know that none of the women of whom the nicotine and cotinine levels were measured underestimated their smoking habits.

The amount of products of tobacco smoke transmitted to the fetus varies consistently, depending on the proportion of each cigarette consumed, frequency of puffing, depth of inhalation and maternal metabolism.[14,15] After birth, the neonates metabolism affects levels and duration of exposure to smoke products. For this reason, cotinine measurements were carried out in neonates of smokers and non-smokers and in neonatal plasma in parallel to maternal plasma. The results of this study show that cotinine is easily transferred to the neonatal compartment. Cotinine concentrations were significantly lower in the neonates than in their mothers ($p< 0.01$), but there was a strong positive linear relationship between maternal and neonatal cotinine concentrations ($r_s= 0.912$, $p< 0.001$). Donnenfeld et al[26] found that fetal cotinine concentrations were about 90% of maternal values throughout gestation. Luck et al[27] found similar cotinine concentrations in smoking mothers and their neonates.

The comparison between neonates of smoking and non-smoking mothers showed cotinine concentrations below the detection limit of 10 µg/ml in neonates of non-smoking mothers and in neonates of mothers who smoked less than 10 cigarettes/ day. Infants of mothers who smoked ≥ 10 cigarettes/day had significantly higher cotinine levels ($p< 0.001$). There seems to be a threshold around 10 cigarettes/day.

Bardy et al[28] measured tobacco exposure during pregnancy to study neonatal effects in relation to maternal smoking. They found a quantitative dose and effect relation between tobacco exposure (cotinine concentrations) and a decrease in the gestational age at birth and the size of the neonate. It might be a coincidence that Peacock et al[29] found a threshold of 13 cigarettes per day for the effect of smoking on birth weight.

In conclusion, cotinine is more useful than nicotine in discriminating non-smokers, light and heavy smokers. Cotinine, the principal metabolite of nicotine is easily transferred to the neonate when his/her mother smokes ≥ 10 cigarettes per day. Cotinine concentrations in neonates are significantly lower than in their mothers, but there is a strong positive linear relationship between cotinine concentrations in the maternal and neonatal compartment.

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Chapter 4

Leucocyte count and leucocyte differential in smoking and non-smoking females during pregnancy

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Abstract

The total leucocyte count was studied in 194 smoking and 518 non-smoking healthy pregnant women. Smoking in pregnancy appeared to have an additive enhancing effect on the already known total leucocyte increase in pregnancy. The leucocyte differential count determined in a smaller group of 105 smoking and 288 non-smoking pregnant women, showed that the eosinophil and basophil count was not involved in the white blood cell shift. The rise of the total leucocyte count was mainly due to an increase of neutrophils, monocytes and lymphocytes. The leucocytosis in the smoking pregnant women was dose-related: significant upward jumps of the percentages of leucocytosis were observed between 12 and 15 cigarettes/day as well as between 19 and 20 cigarettes/day. Further investigation as to the relevance of these observations for pregnancy and fetal well being has to be conducted.

The total white cell count has been described to rise in pregnancy, due to an increase in neutrophil polymorphonuclear leucocytes.[1] The neutrophil count also rises at the time of the oestrogen peak of a normal menstrual cycle, and if fertilization has occurred the neutrophils continue to rise.[2] They reach a peak at 30 weeks and a plateau during the third trimester.[3] The mean total white cell count is then around 9.0 x 10^9/l, the mean neutrophil count about 6.6 x 10^9/l.[1]
The various reports on monocyte count in pregnancy are not unanimous. Hawes et al[4] found an increase of monocytes in pregnancy, others reported that the monocyte count does not change.[5,6] The lymphocyte count is said not to alter significantly during pregnancy.[3] An elevation of the leucocyte count has also been reported as a consequence of habitual smoking and has been attributed mainly to monocyte release.[7-9] Aim of this study was to investigate whether smoking in pregnancy has an additive effect on the already known leucocyte enhancing effect of each separately. The leucocyte differential count was conducted simultaneously in a smaller group of women to assess which cell kind is predominantly involved in a possible white blood cell shift. Finally the percentual occurrence of leucocytosis in relation with the number of smoked cigarettes per day was established.

Subjects and methods

A total of 518 non-smoking and 194 smoking pregnant women consecutively attending the department of gynaecology and obstetrics of the De Wever Hospital, Heerlen, The Netherlands between july 1992 and april 1993, for monitoring of their pregnancy were included in the study. Exclusion criteria were a diastolic blood pressure ≥90 mmHg, an endocrine disease, a coagulation disorder or medication known to interfere with the haemostatic system. Parous women with a history of (pre)eclampsie, hypertension, diabetes, a coagulation disorder, solutio placentae, immature or premature delivery

and/or a baby small for gestational age were excluded from the study. Twenty percent of the non-smokers and 17% of the smokers used ferro medication. The known duration of the gestation was based upon the last menstrual date and confirmed by ultrasound determinations between 8 and 14 weeks. The number of cigarettes per day was an estimation of the patient at the intake i.e. at the gestational age at which the leucocyte count was measured, in most cases confirmed by her partner. The basic characteristics of these patients are given in Table 1. The leucocyte count was conducted in all patients. The leucocyte differential was counted in a smaller group of 288 non-smoking and 105 smoking pregnant women, after the changes in the leucocyte count became clear. For the evaluation they were ranked in four groups according their gestational age: 0-10 weeks, 11-20 weeks, 21-30 weeks and 31-40 weeks of gestation.
Blood samples were drawn between 8.30 and 9.30 a.m. The samples were drawn into EDTA-K_2 containing tubes (Sarstedt, Nümbrecht, Germany) and kept at room temperature for maximally 5 hours before they were run on the Sysmex NE-8000 (TOA Medical Electronics Corp., Kobe, Japan).
The significance of the differences of the median values of the various groups was assessed by the Mann-Whitney-Wilcoxon test. The significance of the percentages of patients with leucocytosis was tested by the Chi-square method.

Table 1. Basic characteristics of the 288 non-smoking and 105 smoking pregnant women

Groups of patients	Age (years)[a]	Gestation (days)[a]	Number of cigarettes per day[a]	Frequency %	Birthweight (g)[b]	Placental weight (g)[b]
Nulliparous women Non-smokers	30 (27-33)	184 (82-227)	0	44.3	3400 (398)	543 (244)
Smokers	29 (25-32)	168 (70-224)	10 (5-15)	45.7	3119 (534)	506 (128)
Parous women Non-smokers	30 (27-33)	184 (82-227)	0	55.6	3607 (458)	565 (192)
Smokers	29 (25-32)	168 (70-224)	10 (5-15)	54.2	3340 (553)	547 (145)

[a] values represent median (IR); [b] values represent mean (SD).

Results

Table 2 shows the comparison of the white blood cell and differential count in smoking and non-smoking women during normal pregnancy. The median leucocyte, granulocyte, lymphocyte and monocyte counts were significantly higher in smokers than in non-smokers.
In Table 3-5 the course of the granulocytes, lymphocytes and monocytes is given from the beginning of pregnancy until the end in four steps (0-10, 11-20, 21-30, 31-40 weeks) in the smoking and non-smoking groups.
Fig 1 shows that the percentage of patients with a leucocytosis (i.e. leucocyte values above 10 x 10^9/l) varied between 61-63% at a smoking quantity of 3-12 cigarettes per day. Between 12 and 15 cigarettes/day this percentage rose significantly to about 70% (p= 0.02), whereas at a smoking quantity of more than 20 cigarettes/day leucocytosis percentages of 82 and 83% were assessed, which was significantly higher than the 70% at 19 cigarettes per day (p< 0.0001).

Table 2. Differences of the median leucocyte and differential counts tested with the Mann-Whitney-Wilcoxon test

	Normal pregnancy, non-smokers [a]	Normal pregnancy, smokers [a]	Significance p-value
Sample size	518	194	—
Leucocytes (10^9/l)	9.1	10.7	< 0.00001
	(7.7 - 10.5)	(8.8 - 12.5)	
Sample size	288	105	—
Granulocytes	7.04	7.45	0.02
	(5.71 - 8.25)	(6.28 - 9.24)	
Lymphocytes	1.74	2.06	< 0.00001
	(1.47 - 2.04)	(1.66 - 2.59)	
Monocytes	0.42	0.46	0.003
	(0.30 - 0.54)	(0.39 - 0.58)	
Eosinophils	0.10	0.11	n.s.
	(0.07 - 0.15)	(0.08 - 0.16)	
Basophils	0.03	0.04	n.s.
	(0.02 - 0.03)	(0.02 - 0.05)	

[a] values represent median (IR).

Table 3. Comparison of the granulocyte counts of the smoking (S) and non-smoking (NS) females at four stages during pregnancy

	GRANULOCYTES							
Gestational age (weeks)	0-10		11-20		21-30		31-40	
Patient groups	1 NS	2 S	3 NS	4 S	5 NS	6 S	7 NS	8 S
Number	50	30	39	18	76	26	123	31
Median (10^9/l)	5.52	6.16	6.61	6.94	7.02	8.00	7.83	8.49
Lower quartile	4.73	5.25	4.97	5.60	6.08	6.80	6.46	7.85
Upper quartile	6.38	7.18	8.10	8.44	7.92	9.81	8.83	10.60
p-value of Mann-Whitney-Wilcoxon-test	0.09		0.48		0.007		0.003	

Table 4. Comparison of the lymphocyte counts of the smoking (S) and non-smoking (NS) females at four stages during pregnancy

	LYMPHOCYTES							
Gestational age (weeks)	0-10		11-20		21-30		31-40	
Patient groups	1 NS	2 S	3 NS	4 S	5 NS	6 S	7 NS	8 S
Number	50	30	39	18	76	26	123	31
Median (10^9/l)	1.78	2.36	1.81	1.87	1.63	1.86	1.74	2.22
Lower quartile	1.49	1.87	1.46	1.43	1.40	1.66	1.49	1.70
Upper quartile	2.16	2.95	2.09	2.25	1.86	2.07	2.06	2.71
p-value of Mann-Whitney-Wilcoxon-test	0.0001		0.79		0.008		<0.0001	

Table 5. Comparison of the monocyte counts of the smoking (S) and non-smoking (NS) females at four stages during pregnancy

	MONOCYTES							
Gestational age (weeks)	0-10		11-20		21-30		31-40	
Patient groups	1 NS	2 S	3 NS	4 S	5 NS	6 S	7 NS	8 S
Number	50	30	39	18	76	26	123	31
Median (10^9/l)	0.39	0.48	0.39	0.53	0.41	0.45	0.46	0.43
Lower quartile	0.31	0.42	0.34	0.45	0.31	0.35	0.35	0.38
Upper quartile	0.48	0.57	0.50	0.67	0.53	0.60	0.58	0.54
p-value of Mann-Whitney-Wilcoxon-test	0.006		0.02		0.18		0.90	

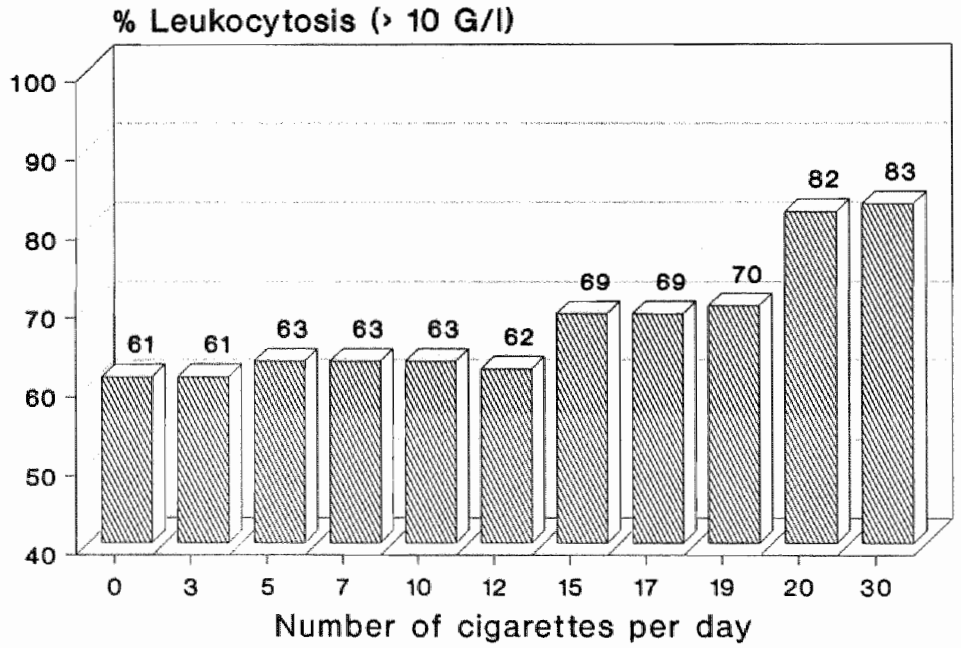

Fig 1. Cumulative frequency of leucocytosis (i.e. leucocyte count > 10 x 10^9/l) with increase of the number of cigarettes per day

Discussion

The present study was designed prospectively and has to be seen as an initial study to test the impact of smoking on the leucocytes during pregnancy. Various reports have established that total white blood cell counts are significantly higher in smokers compared with non-smokers.[10-12] Effects of smoking on the haematological system have been reported in the Caerphilly and Speedwell Collaborative Surveys, a study on risk factors for ischemic heart disease.[7] Haematocrit and white cell count were found to be much higher in smokers than in ex-smokers and non-smokers. Dose response relations were apparent in current smokers in terms of white cell count, packed and mean cell volumes. Smoking in pregnancy has an additive effect on the already known leucocyte increase in pregnancy. The reason for the leucocytosis in pregnancy is not known. Although it is difficult to find an explanation for the fact that smoking increases the number of leucocytes in pregnancy, the consequences of these changes might be of importance. The leucocyte differential count shows that the eosinophil and basophil count is not involved in the white blood cell shift. The increase in total leucocyte count is mainly due to an increase in neutrophils, monocytes and lymphocytes. Neutrophils are necessary in the body's first reaction to micro-organisms, whereas monocytes and lymphocytes (T and B cells) are part of the humoral and cell mediated immunity. Damm et al[13] studied the distribution of peripheral T cells, monocytes/granulocytes, natural killer cells and B cells in aborting women. They established that the normal pregnancy has no influence on the distribution of mononuclear cells. In cases with threatening abortions the number of monocytes/granulocytes and natural killer cells was increased if the loss of pregnancy occurred. It is not clear whether the leucocytosis is a reaction to the abortion or vice versa. Cell mediated immunity is depressed in normal pregnancy in order to be able to accept the fetal allograft. Moncharmont et al[14] conducted a study to find out whether lymphocyte subsets are modified by pregnancy. T lymphocyte subsets,natural killer cells and CD11a+ cells decreased during pregnancy. The authors stated that these findings partially explain the tolerance for the fetus. Leucocyte metabolism is related to fetal growth.[16-19] Metcoff et al[19] demonstrated a decreased activity of the enzymes adenylate kinase and pyruvate kinase in leucocytes of the cord blood and in maternal leucocytes in children with intrauterine growth retardation. Carboné et al[18] studied maternal leucocyte metabolism in pregnancy. The results of their study indicate that the energy metabolism, as measured by enzyme activities and adenine nucleotide levels, increases during the first half of pregnancy in women who deliver fullterm babies with a normal weight.

There is no doubt that smoking has an effect on pregnancy outcome. Simpson reported as early as 1957 that smoking during pregnancy increases the risk of premature birth.[20] The association between smoking and perinatal death is disputed.[21] An effect of smoking on birth weight is a constant finding. Most studies have found a dose dependent effect of smoking on birth weight.[22] Peacock[23] found a threshold for the effect of

smoking on birth weight. The threshold level was 13 cigarettes. In the present study a break-point in the percentage of leucocytosis is found at 12 cigarettes per day, which might be fortuitous, but it is congruent with the results of Peacock.

The effect on intrauterine smoke exposure of the child is not only found in a reduction of birth weight. Several studies have reported an association between maternal smoking and pulmonary morbidity of her offspring.[24] Children exposed to maternal smoking in utero are said to have significant more neuropsychological deficits than children who are not exposed.[25] Two studies indicate a clear dose-related increase in cancer risk in children of mothers who smoked in pregnancy, which was particularly marked for acute leukaemias.[26,27]

In conclusion, the effects of smoking in pregnancy are numerous and the leucocyte enhancement is one of them. Further investigations, however, have to be conducted to establish whether this effect is adverse for pregnancy and fetal well-being.

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Chapter 5

Erythrocyte count and indices during normal pregnancy of non-smoking and smoking women

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Abstract

In 247 non-smoking and 123 smoking healthy pregnant women the erythrocyte count and indices were compared at four different stages of pregnancy: 0-10, 11-20, 21-30 and 31-40 weeks. Exclusion criteria were a diastolic pressure ≥ 90 mmHg, an endocrine disease or a coagulation disorder. A women was considered a smoker if she smoked 4 or more cigarettes/day. Blood samples were run on a Sysmex NE-8000.
The erythrocyte count was significantly lower in smokers than in non-smokers (3.86×10^{12}/l versus 3.96×10^{12}/l) in the last ten weeks. Comparing the erythrocyte count during the beginning and the end of pregnancy there were significant lower values in both groups (4.32×10^{12}/l to 3.96×10^{12}/l in the non-smoking and 4.24×10^{12}/l to 3.86×10^{12}/l in the smoking group). The differences in the median HGB and HCT levels were neglectable. The MCV was significantly higher in women who smoked, as was the MCH (MCV 91 fl and MCH 1.90 fmol in the non-smoking versus MCV 94 fl and MCH 1.95 fmol) in the smoking group in the last ten weeks.
Smoking in pregnancy leads to a lower erythrocyte count and a higher MCV which might create a hypoxic condition of the fetus.

Plasma volume and total red cell mass are under separate control and bear no fixed relation to each other. Changes in pregnancy provide an illustration of this point. Plasma volume rises progressively throughout pregnancy to a plateau in the last 8 weeks.[1] The increase in plasma volume is correlated with the birth weight of the baby.[2] The red cell mass, which represents the total volume of red cells in the circulation, also rises during normal pregnancy. Because the increase in red cell mass during pregnancy is proportionately less than the increase in plasma volume the concentration of red cells in the blood declines. The size and haemoglobin content of the cells show only minor changes; consequently the haemoglobin concentration and haematocrit fall parallel with the red cell count. Little has been reported on the effect of chronic cigarette smoking on the erythrocytic system. It may decrease vitamin B_{12} which could lead to an elevation of the mean corpuscular volume (MCV).[3] Heavy long-term smoking may cause an erythrocytosis.[4] Cigarette smoking is also associated with an enhanced haemoglobin-CO concentration causing a mild form of hypoxia.[5] The haemoglobin synthesis might also be impaired by the lead content of tobacco.[6] Finally, smoking increases the plasma viscosity.[7] Even less is known about the haematological consequences of cigarette smoking in pregnancy. The aim of this study was to compare the erythrocyte count and the erythrocyte indices of smoking and non-smoking women at different stages of normal gestation.

Subjects and methods

A total of 247 non-smoking and 123 smoking healthy pregnant women were included in this cross-sectional study. These women consecutively attended the obstetrical department of the De Wever Hospital, Heerlen, The Netherlands between november 1992 and april 1993. Exclusion criteria were a diastolic pressure ≥ 90 mmHg, an endocrine disease or a coagulation disorder. The duration of the gestation was based on the last menstrual date and an ultrasound determination between 8 and 14 weeks. The number of cigarettes per day was an estimation of the patient, in most cases confirmed by her partner. A women was considered a smoker if she smoked 4 or more cigarettes/day. The basic characteristics of these patients are given in Table 1.
Blood samples were drawn between 8.30 and 9.30 a.m. into EDTA-K_2 containing tubes (Sarstedt, Nümbrecht, Germany) and kept at room temperature for maximally 5 hours before they were run on a Sysmex NE-8000 (TOA Medical Electronics Corp., Kobe, Japan).
The significance of the differences of the median values of the various groups was assessed by the Mann-Whitney-Wilcoxon test.

Results

In Table 2 the erythrocyte count, the HGB concentration, the HCT values and the MCV and MCH values of the smoking and non-smoking group during the gestational period are shown. Significantly lower values of the erythrocyte count were found in women who smoked during pregnancy. However, they did not start with a lower erythrocyte count, as the difference in the first ten weeks of pregnancy was not significant (4.32 x 10^{12}/l in the non-smoking versus 4.24 x 10^{12}/l in the smoking group).

Table 1. Basic characteristics of the pregnant women

Groups of patients		Parity	Age (years)[a]	Iron medic	Cigarettes per day [a]
Non-smokers, normal pregnancy	(n=247)	47.9% primi 52.1% multi	30 (27-33)	20%	0
Smokers, normal pregnancy	(n=123)	47.8% primi 52.2% multi	29 (25-32)	17%	10 (5-15)

[a] values represent median (IR).

Table 2. Comparison of the erythrocyte counts, the HGB concentration, the HCT values, the MCV and MCH values of the smoking (S) and non-smoking (NS) females at four stages during normal pregnancy[a]

	Gestational age (weeks)							
	0-10		11-20		21-30		31-40	
	NS	S	NS	S	NS	S	NS	S
Number	93	46	66	29	119	45	180	68
Erythrocyte count								
Median (10^{12}/l)	4.32[1)]	4.24[1)]	4.19[2)]	3.88[2)]	3.88[3)]	3.69[3)]	3.96[4)]	3.86[4)]
Lower quartile	4.08	4.07	3.85	3.77	3.67	3.53	3.75	3.67
Upper quartile	4.56	4.44	4.40	4.11	4.13	3.86	4.20	4.04
p-value of MWW-test	[1)] : 0.21		[2)] : 0.002		[3)] : 0.0004		[4)] : 0.017	
Haemoglobin concentration								
Median (mmol/l)	8.2[1)]	8.4[1)]	7.9[2)]	7.4[2)]	7.4[3)]	7.3[3)]	7.5[4)]	7.5[4)]
Lower quartile	7.7	7.9	7.4	7.2	7.0	6.7	7.1	7.2
Upper quartile	8.4	8.7	8.2	8.0	7.9	7.6	7.9	7.8
p-value of MWW-test	[1)]: 0.09		[2)]: 0.053		[3)]: 0.063		[4)]: 0.81	
Haematocrit								
Median (%)	38.6[1)]	39.3[1)]	37.4[2)]	35.6[2)]	35.9[3)]	35.3[3)]	36.2[4)]	36.0[4)]
Lower quartile	37.7	38.2	35.1	35.0	34.0	32.7	34.5	34.6
Upper quartile	40.1	41.3	39.0	38.7	37.6	36.2	37.6	37.6
p-value of MWW-test	[1)]: 0.09		[2)]: 0.15		[3)]: 0.034		[4)]: 0.96	
MCV								
Median (fl)	90[1)]	93[1)]	90[2)]	93[2)]	92[3)]	93[3)]	91[4)]	94[4)]
Lower quartile	87	91	87	91	89	91	88	90
Upper quartile	92	95	93	96	95	97	94	96
p-value of MWW-test	1) <0.0001		[2)] <0.0001		[3)] 0.046		[4)] 0.0013	
MCH								
Median (fmol)	1.89[1)]	1.97[1)]	1.88[2)]	1.94[2)]	1.94[3)]	1.96[3)]	1.90[4)]	1.95[4)]
Lower quartile	1.84	1.88	1.82	1.90	1.84	1.88	1.81	1.86
Upper quartile	1.94	2.01	1.98	1.97	2.00	2.01	1.96	2.02
p-value of MWW-test	[1)] 0.0003		[2)] 0.043		[3)] 0.14		[4)] 0.006	

MWW = Mann-Whitney-Wilcoxon-test; [a] abbreviations are mentioned in text; 1-4 represent the four stages of pregnancy.

Table 3. Survey of the data on the pregnancy outcome of 123 smokers and 247 non-smokers

Groups of patients	Frequency %	Birth weight[a] (g)	Placental weight[a] (g)	Fluxus post partum[b] (cc)	Thrombosis post partum[b]
Nulliparous women					
Non-smokers	47.9	3400 (398)	543 (244)	4	0
Smokers	47.8	3119 (534)	506 (128)	3	0
Parous women					
Non-smokers	52.1	3607 (458)	547 (145)	4	0
Smokers	52.2	3340 (553)	565 (192)	2	0

a values represent mean (SD); b values represent incidence.

Comparing the erythrocyte count during the first and last 10 weeks of pregnancy there were significant lower values in both groups (4.32 x 10^{12}/l to 3.96 x 10^{12}/l in the non-smoking and 4.24 x 10^{12}/l to 3.86 x 10^{12}/l in the smoking group). The Hb levels of the smoking group were lower throughout gestation, but the differences were not significant. The differences between the median HCT values of the smokers and non-smokers were neglectable. The MCV was significantly higher in smokers (94 fl versus 91 fl in the last ten weeks). This also applies for the MCH (1.95 fmol versus 1.90 fmol in the last ten weeks). Smokers started with higher MCV and MCH levels but these parameters did not rise further throughout pregnancy.

Discussion

In the group of chronic cigarette smokers the erythrocyte count during the gestational period was lower and both the mean corpuscular volume and the mean corpuscular haemoglobin were higher. Moreover, there seemed to be a lower haemoglobin concentration in the women who smoked but that difference was not significant. The haematocrit level was not altered by smoking. Whether these haematological changes have an adverse effect on the course of the gestation and the growth of the fetus might be questioned. A lower erythrocyte count is equiva-lent with fewer oxygen transporting particles, which might create a hypoxic condition for the fetus. Increased haemoglobin

and haematocrit levels have been reported in newborns of smoking mothers.[8] An explanation could be that maternal smoking creates a hypoxic condition for the fetus and thus stimulates erythropoiesis.

Smoking has been described to enhance blood viscosity.[9] In pregnancy whole blood viscosity decreases significantly from an amenorrhea of 16 weeks to 34 weeks, after which this tendency reverses.[10] Pregnancies with intrauterine growth retardation have been reported to have elevated whole blood viscosity compared with non-affected pregnancies.[11] Nyland et al[12] found a significantly decreased utero-placental perfusion in women delivering a growth-retarded child. Maternal blood viscosity might influence fetal growth by affecting placental perfusion on the maternal side.[13] An enhancement of the blood viscosity provokes a reduction of the MCV. However, in the patients who smoked there was an elevation of the MCV. A lower maternal erythrocyte count and higher maternal MCV is not a favourable combination for the fetus. The production of PGI_2 in the umbilical cord is decreased by smoking which causes a constriction of the vessels in the placenta. A lower erythrocyte count means less oxygen transport whereas the transport of larger erythrocytes is more difficult in constricted vessels. The haemoglobin concentration was decreased in pregnant women who smoked. High maternal haemoglobin levels, reflecting a comparatively decreased plasma volume, have been associated with uterine fetal death.[14-17] On the opposite, low maternal haemoglobin levels have been found in mothers with large newborns.[18] In our study group, neonates and placentas of (nulli) parous smokers weighed significantly less than neonates and placentas of non-smokers (Table 3).

In conclusion, smoking in pregnancy leads to a lower erythrocyte count and a higher MCV. Although we are aware that the shifts remain within the reference range, these findings might create a slightly hypoxic condition for the fetus. Further investigation has to be done on the erythrocyte count and indices in umbilical cord blood to assess the relevance of these data for newborns who have been exposed to maternal smoking compared to those who have not been exposed.

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Chapter 6

The reticulocyte count and its subfractions in smoking and non-smoking pregnant women

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Abstract

Our objective was to compare the reticulocyte count and its subfractions in smoking and non-smoking women at different stages of normal pregnancy. The reticulocyte count and its subfractions were compared in 247 non-smoking and 123 smoking healthy pregnant women at four different stages of pregnancy: 0-10, 11-20, 21-30 and 31-40 weeks. Exclusion criteria were a diastolic pressure ≥ 90 mmHg, an endocrine disease or a coagulation disorder. Women in the smokers group smoked more than 4 cigarettes/day. Non-smokers were defined as women reporting no smoking at all. Blood samples were run on a Sysmex R-3000 reticulocyte counter. The absolute reticulocyte count was lower in the smoking group throughout pregnancy, but this was only significant in the last ten weeks of gestation (71.9×10^9/l versus 78.8×10^9/l). There was no difference between the low fluorescence, the medium fluorescence and the high fluorescence proportions in the non-smoking and the smoking group. Both groups behaved similarly during pregnancy; there was a decrease of mature reticulocytes and a significant increase of more immature reticulocytes.
These data show a moderate measurable effect of cigarette smoking on the reticulocyte count and the absence of an effect on the reticulocyte subsets.

Reticulocyte counting allows the direct measurement of the activity of erythropoiesis in the bone marrow. Manual reticulocyte counting has been reported as problematic.[1-5] To improve the reliability of reticulocyte counting, several automated methods and instruments have been introduced.[6-8] The Sysmex R-3000 was used in this study. This instrument is able to provide precise and statistically reliable reticulocyte counts.[9] Furthermore, it estimates the maturity of a reticulocyte by measuring the fluorescence intensity, a reflection of the RNA content of the cell. Castriota et al[10] studied the total reticulocyte count and the reticulocyte fluorescence intensity ratios in a normal paediatric population. They found that the reticulocyte subsets allow a more accurate evaluation of bone marrow activity than the total reticulocyte count.
The reticulocyte population can be divided into three groups. They are defined by ratios of the total fluorescence intensity and termed low, medium and high fluorescence ratios.[11] As the reticulocytes become older, their fluorescence (RNA content) decreases. The high fluorescence ratio is therefore a reflection of the most immature reticulocyte. These phenomena have not been investigated in pregnant women. The aim of this study was to compare these quantities in smoking and non-smoking females during a normal pregnancy.

Subjects and methods

Two hundred and forty-seven non-smoking and 123 smoking healthy pregnant women were included in the study. These women consecutively attended the obstetrical depart-

ment of the De Wever Hospital, Heerlen, The Netherlands between november 1992 and april 1993. The reticulocyte count and subsets were determined at four different stages (0-10, 11-20, 21-30, 31-40 weeks) of pregnancy. Not all patients were checked in all four stages. Exclusion criteria were a diastolic pressure ≥ 90 mmHg, an endocrine disease or a coagulation disorder. The duration of the gestation was based on the last menstrual period and an ultrasound determination between 8 and 14 weeks. The number of cigarettes smoked per day was an estimation of the patient, in most cases confirmed by her partner. If a women smoked more than 4 cigarettes/day she was called a smoker. The group of women who smoked more than 15 cigarettes/day was too small to subdivide the smokers into an intermediate smoking level group (5-15 cigarettes per day) and a group of heavy smokers (> 15 cigarettes per day). Non-smokers were defined as women reporting no smoking at all. The basic characteristics of these patients are given in Table 1.

Venous blood samples were collected in EDTA-K_2 containing tubes (Sarstedt, Nümbrecht, Germany). Samples were kept at room temperature until analysis. The measurements were performed with the relatively new Sysmex R-3000 reticulocyte counter (Toa Medical Electronics, Kobe, Japan). The coefficients of the interassay variation for the reticulocyte count amounted 6.7% and 7.6% at levels of 14 x 10^9/l and 36 x 10^9/l respectively (reference range for women 20-110 x 10^9/l). This instrument provides the reticulocyte count as a percentage of the erythrocytes, the absolute reticulocyte count, the red blood cell count and the platelet count as well as a cytogram from which the reticulocytes can be subdivided into low, medium and high fluorescence ratio reticulocytes, in that order indicating three stages in the maturation of the reticulocytes. The significance of the differences of the median values of the various groups was assessed by the Mann-Whitney-Wilcoxon test.

Table 1. Basic characteristics of the pregnant women

Groups of patients	Age[a] (years)	Iron medication	Parity	Cigarettes per day[a]
Non-smokers, normal pregnancy (n=247)	30 (27 - 33)	20 %	47.9% primi 52.1% multi	0
Smokers, normal pregnancy (n=123)	29 (25 - 32)	17 %	47.8% primi 52.2% multi	10 (5-15)

[a] Values represent median (IR).

Results

Figure 1 gives a comparison of the median values of the absolute reticulocyte count of smoking and non-smoking females in four stages of pregnancy.
The reticulocyte count throughout pregnancy was lower in the smoking group but this was only significant in the 31-40th week of gestation (71.9 x 10^9/l versus 78.8 x 10^9/l; $p< 0.014$).
The median low, medium and high fluorescence ratio percentages did not differ significantly between the two groups. The three reticulocyte subfractions behaved similarly in both groups. The low fluorescence ratio decreased (from 88.5 to 78.6% in the non-smoking, from 88.3 to 77.4% in the smoking group), whereas the medium fluorescence ratio (from 10.7 to 18.0% in the non-smoking, from 10.8 to 17.4% in the smoking group) and the high fluorescence ratio (from 1.0 to 3.6% in the non-smoking, from 1.1 to 4.4% in the smoking group) increased significantly from the beginning to the end of gestation.

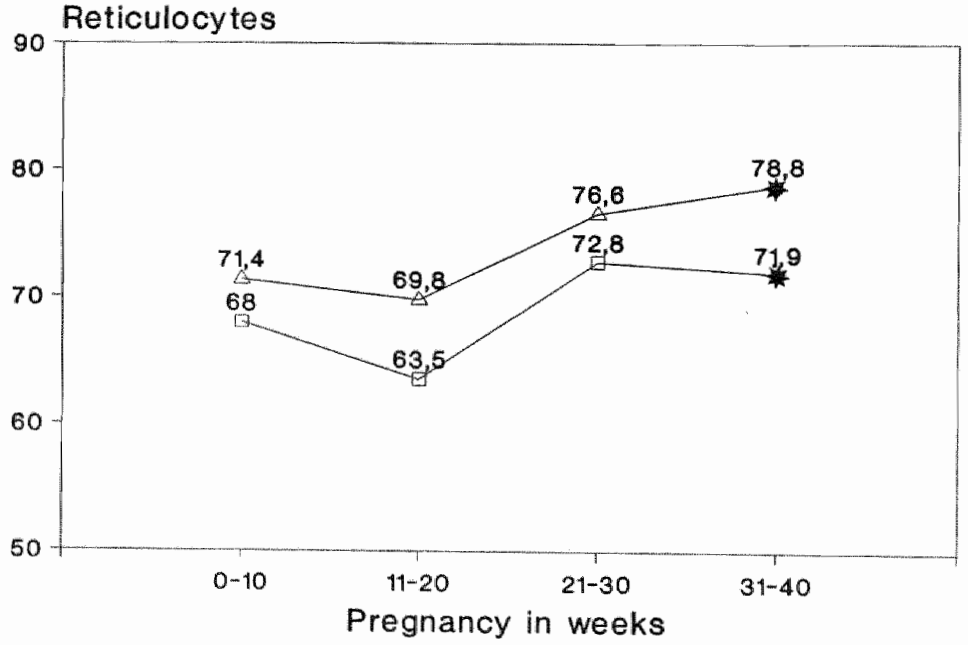

Fig 1. Comparison of the absolute values of the reticulocyte count of the smoking (□) and non-smoking (Δ) females in four stages of pregnancy. Significant difference is indicated by an asterisk (✱).

Discussion

The erythrocyte count and indices in smoking and non-smoking pregnant women were studied earlier.[12] Smoking pregnant women appeared to have lower erythrocyte counts from the 11th week of gestation onwards. An increase of bone marrow activity and a consequent increase of reticulocytes was to be expected in the same period. In the present study however, smoking pregnant women had a lower reticulocyte count in the last ten weeks of pregnancy, suggesting an absence of increased bone marrow activity. The reticulocyte subsets did however show a significant increase of bone marrow activity, both in non-smoking and in smoking pregnants. The low fluorescence ratio, indicating the more mature reticulocytes, decreased, whereas the medium and high fluorescence ratios, representing the more immature reticulocytes, increased significantly from the beginning to the end of gestation. The relative stability of the reticulocytes and the steady increase of the reticulocyte subsets suggest a rapid decrease of the intra-erythrocytic RNA content in the course of the reticulocyte maturation during pregnancy. This process seems to be independent whether or not the pregnant woman smokes. The latter is remarkable as one would expect a counterregulation of erythropoiesis as a reaction to the decline of erythrocytes due to smoking in pregnancy.

Cigarette smoke may have an effect on the bone marrow and thus affect erythropoiesis. German[13] found an inhibition of erythropoiesis: in individuals who smoked less than five years the reticulocyte maturation rate was reduced, as was the level of circulating red cells. Controversially, the erythropoiesis was normalized in those subjects who smoked more than five years. The author stated that these findings reflect different phases of the toxic effects of tobacco smoke on the bone marrow.

However, cigarette smoke may also have a direct effect on the erythrocyte itself. The same investigator found a toxic effect on the red cell. Six to ten years of smoking resulted in toxic lesions of the circulating erythrocytes confirmed by an increase of the number of spherulation altered cells, early onset and a late termination of haemolysis and an increase of the percentage of perished cells.[14] The biological mechanism is unclear.

Conclusion

The measurable effect of cigarette smoking on the reticulocyte count was moderate, whereas the effect on the low, medium and high fluorescence proportions was absent. These results indicate that no real counterregulation occurs as a reaction to the decline of erythrocytes due to smoking in pregnancy. The mechanisms by which smoking affects the bone marrow or the erythrocyte itself remains unclear.

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Chapter 7

Platelet count and platelet indices at various stages of normal pregnancy in smoking and non-smoking women

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Abstract

Our objective was to compare the platelet count and platelet indices of smoking and non-smoking women at different stages of normal pregnancy. In 247 non-smoking and 123 smoking healthy pregnant women the platelet count, the mean platelet volume, the platelet distribution width and the plateletcrit were compared at 0-10, 11-20, 21-30 and 31-40 weeks of pregnancy. Exclusion criteria were a diastolic pressure ≥ 90 mmHg, an endocrine disease, a coagulation disorder, acetylsalicylic acid or phenprocoumon use. A women was considered a smoker if she smoked more than 4 cigarettes a day. Non-smokers were defined as women reporting no smoking at all. Blood samples were run on a Sysmex NE-8000.
There was no significant difference between the platelet count in the two groups. In the non-smoking group, the platelet count showed a significant decrease with gestational age (287 x 10^9/l to 258 x 10^9/l). This was not the case in the smokers group. The mean platelet volume of the smokers was significantly lower than that of the non-smokers in the last ten weeks of pregnancy (10.4 fl versus 10.7 fl). The platelet distribution width and the plateletcrit did not change under the influence of cigarette smoking.
Smoking during pregnancy does not affect platelet count or platelet indices.

In recent years there has been a broad interest in defining the mechanisms responsible for the adverse effects of cigarette smoking.[1-3] Various reports have focused on the influence of smoking on platelets because of a possible association between smoking, an alteration of blood platelets and atherosclerosis.[4-7] Some of these studies showed an increase of platelet turnover and a decrease of platelet survival in smokers.[4,7] The increased destruction of platelets, however, was not sufficient to reduce the number of circulating platelets. Platelet counts either remained normal[4,8] or were increased.[9,10] Smoking and platelet behaviour during pregnancy were not addressed up to now. Most platelet studies have been confined to platelet counts in normal pregnancy regardless of the smoking behaviour of the women, although the combination of platelet and platelet size parameters as mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) might provide better insight in platelet biology during pregnancy. The aim of this study was to assess the platelet count and platelet indices at various stages of normal pregnancy in smoking and non-smoking women.

Subjects and methods

Two hundred and forty seven non-smoking and 123 smoking healthy pregnant women consecutively attending the obstetrical department of the De Wever Hospital, Heerlen, The Netherlands between november 1992 and april 1993 for monitoring of their pregnancy, were included in the study. The platelet count and platelet indices were determined at four different stages (0-10, 11-20, 21-30, 31-40 weeks) of pregnancy.

Not all patients were checked in all four stages. Exclusion criteria were a diastolic pressure ≥ 90 mmHg, an endocrine disease or a coagulation disorder. None of the patients used acetylsalicylic acid or phenprocoumon. The known duration of the gestation was based on the last menstrual period and an ultrasound determination between 8 and 14 weeks. The number of cigarettes smoked per day was estimated by the patient, in most cases confirmed by her partner. A women was considered a smoker if she smoked more than 4 cigarettes a day. The group of women who smoked more than 15 cigarettes a day was too small to subdivide the smokers into an intermediate smoking level group (5-15 cigarettes per day) and a group of heavy smokers (> 15 cigarettes per day). Non-smokers were defined as women reporting no smoking at all. The basic characteristics of these patients are given in Table 1.
Blood samples were drawn between 8.30 and 9.30 a.m. into EDTA-K_2 containing tubes (Sarstedt, Nümbrecht, Germany) and kept at room temperature for at most 5 hours before they were run on the Sysmex NE-8000 (TOA Medical Electronics Corp., Kobe, Japan). The inter-assay coefficients of variation amounted for the platelet count 5.9% at 259 x 10^9/l (assigned value 253 x 10^9/l), for the mean platelet volume 1.8% at 8.8 fl (assigned value 8.4 fl), for the platelet distribution width 2.8% at 9.5 % and for the plateletcrit 18 x 10^{-4} l/l at 22 x 10^{-4} l/l.
The significance of the differences of the median values of the various groups was assessed by the Mann-Whitney-Wilcoxon test.

Results

In Table 2 the comparison of the platelet counts of the smoking and non-smoking females at four stages of pregnancy is given. In the non-smoking group the platelet count showed a slight but significant decrease with gestational age. The group started with a median platelet count of 287 x 10^9/l and ended with a platelet count of 258 x 10^9/l (p= 0.002). The decrease from 283 x 10^9/l to 264 x 10^9/l in the smokers group however was not significant (p= 0.86).
The MPV of the smokers was significantly lower than the volume of the non-smokers in the last ten weeks of pregnancy (10.4 versus 10.7; p= 0.02). The MPV in the non-smoking group did not change during gestation. This quantity was also more or less stable in the smoking group. The PDW of the two groups was compared and did not change under the influence of smoking. There was also no difference in the plateletcrit values. In the non-smoking group the PDW increased significantly from the beginning to the end of pregnancy (11.7% to 12.3%; p= 0.03). This was not the case in the smoking group. The PCT in the non-smokers decreased significantly throughout pregnancy (26.3 x 10^{-4} l/l to 24.9 x 10^{-4} l/l; p= 0.04). The PCT in the smokers also decreased throughout pregnancy, but this difference was not significant.

Table 1. Basic characteristics of the pregnant women

Groups of patients	Age[a] (years)	Parity	Cigarettes per day[a]
Non-smokers, normal pregnancy (n=247)	30 (27 - 33)	47.9% primi 52.1% multi	0
Smokers, normal pregnancy (n=123)	29 (25 - 32)	47.8% primi 52.2% multi	10 (5-15)

[a] Values represent median (IR).

Table 2. Comparison of the platelet counts of the smoking (S) and non-smoking (NS) females at four stages during normal pregnancy

	PLATELETS							
Gestational age (weeks)	0-10		11-20		21-30		31-40	
Patient groups	NS	S	NS	S	NS	S	NS	S
Sample size	93	46	66	29	119	45	180	68
Median (10^9/l)	287[1)]	283[1)]	264[2)]	267[2)]	264[3)]	283[3)]	258[4)]	264[4)]
Lower quartile	232	215	230	239	226	247	211	232
Upper quartile	325	322	302	299	308	334	302	312
p-value of MWW-test	n.s.		n.s.		n.s.		n.s.	

MWW = Mann-Whitney-Wilcoxon-test; [1-4] represent the four stages of pregnancy.

Discussion

In the present study the only difference in platelet counts observed was a slight but significant decrease with gestational age in the non-smoking group. Platelet survival is known to be diminished in late pregnancy.[11] This is due to an accelerated state of coagulation and fibrinolysis which increases towards term.[12] Haemodilution in pregnancy may be another explanation for the decrease of platelets.[13] It is remarkable that this decrease of platelets does not occur in the smoking group.

Most platelet studies in normal pregnancy have been confined to platelet counts, but the platelet count alone is not conclusive. Platelet size indices provide more information about platelet biology. The MPV is an indication of the amount of young platelets. Fay et al[14] studied platelet count and indices in blood samples from 2066 healthy women with an uncomplicated pregnancy. The MPV remained stable until 35 weeks gestation and rose dramatically thereafter. Ahmed et al[15] studied the MPV in healthy pregnant women. This quantity remained constant between the first trimester and the end of normal pregnancy. In patients who became pre-eclamptic a persistent increase in MPV was found. Hutt et al[16] found that the mean platelet volume increased 2-3 weeks before the development of pre-eclampsia.

In our study the MPV was significantly lower in the last ten weeks of pregnancy in the smoking than in the non-smoking group. The MPV of the non-smokers remained almost constant from the beginning to the end of pregnancy.

Less has been published regarding PDW and PCT in pregnancy. In the present study these indices remained more or less constant in both smoking and non-smoking women throughout pregnancy. This is in accordance with earlier results of our group[17], when platelet indices were studied in normal pregnancy.

In several studies the effect of the tobacco smoke constituents nicotine and carbon monoxide on platelet aggregation in non-pregnant women has been evaluated in vivo and in vitro.[18-21] They generally showed evidence of platelet activation. Smokers had an enhanced aggregation of platelets, although the platelet count did not differ from non-smokers. The present study indicates that smoking during pregnancy does not affect platelet count and platelet indices in a clinically relevant way.

However, the effect of the combination of smoking and pregnancy on platelet aggregation still has to be assessed.

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Chapter 8

Coagulation and fibrinolysis in smoking and non-smoking pregnant women

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Abstract

The objective of the study was to assess the effects of smoking during pregnancy on coagulation and fibrinolysis. Forty-four non-smoking and 57 smoking pregnant women were included in the study. Prothrombin fragment 1+2 and TAT levels were assessed to monitor coagulation activation. Plasminogen, α_2-antiplasmin and D-dimer levels were determined in order to measure the fibrinolytic activity.
Parameters of coagulation activation increased significantly with gestational age. Prothrombin fragment 1+2 increased from 0.8 nmol/l to 2.5 nmol/l in the non-smoking group of pregnant women and from 1.0 nmol/l to 1.8 nmol/l in the smoking group. Thrombin-antithrombin III levels increased from 2.2 µg/l to 9.9 µg/l in the non-smoking group and from 3.1 µg/l to 8.5 µg/l in the smoking group. Parameters of fibrinolysis showed a different picture. Plasminogen levels in both groups rose significantly in the first half of gestation reaching a plateau in the second half. The α_2-antiplasmin levels remained constant in both groups, although the smokers started with significantly higher levels: 119% versus 105% in the non-smokers. The D-dimer levels rose significantly in both groups: from 278 µg/ml to 847 µg/ml in the non-smokers and from 215 µg/ml to 520 µg/ml in the smokers. They were significantly lower in the smoking group from the 11th up to the 40th week. The D-dimer/TAT ratio was significantly higher in the non-smokers.
In smoking pregnant women the activated coagulation process was not counterbalanced by an adequate increase of fibrinolysis which was the case in the non-smokers.

Coagulation is activated in response to rupture of a vessel or damage to special activator substances in the blood. A complex of substances called prothrombin activator is formed through two different pathways (Fig.1). The extrinsic pathway (factor VII) begins with trauma of the vascular wall or tissue and the intrinsic pathway (factor XII, XI, IX, VIII) starts in the blood itself. In both pathways various plasma proteins play major roles. These blood clotting factors are inactive proteolytic enzymes. When converted to the active forms their enzymatic action causes successive reaction of the clotting process, resulting in the formation of prothrombinase (factor Xa-complex), which converts prothrombin to thrombin. Thrombin activity is regulated by complex formation with antithrombin III, the main physiological inhibitor of blood coagulation. The resulting thrombin-antithrombin III complex (TAT) reflects coagulation activation. Free thrombin leads to the formation of fibrin, which has to be degraded by fibrinolysis.
For this purpose, the inactive enzyme precursor plasminogen is converted into the active protease plasmin by plasminogen activators. Two types of plasminogen activators have been identified: the tissue type (t-PA) and the urokinase type (u-PA). The control of plasminogen activator may occur at the level of synthesis and release, but also through its interaction with specific plasminogen activator inhibitors (PAI). PAI-1, initially called endothelial cell PA inhibitor, is the most important PA-inhibitor in plasma. PAI-2, the placental type inhibitor, appears to have a major role in the control of

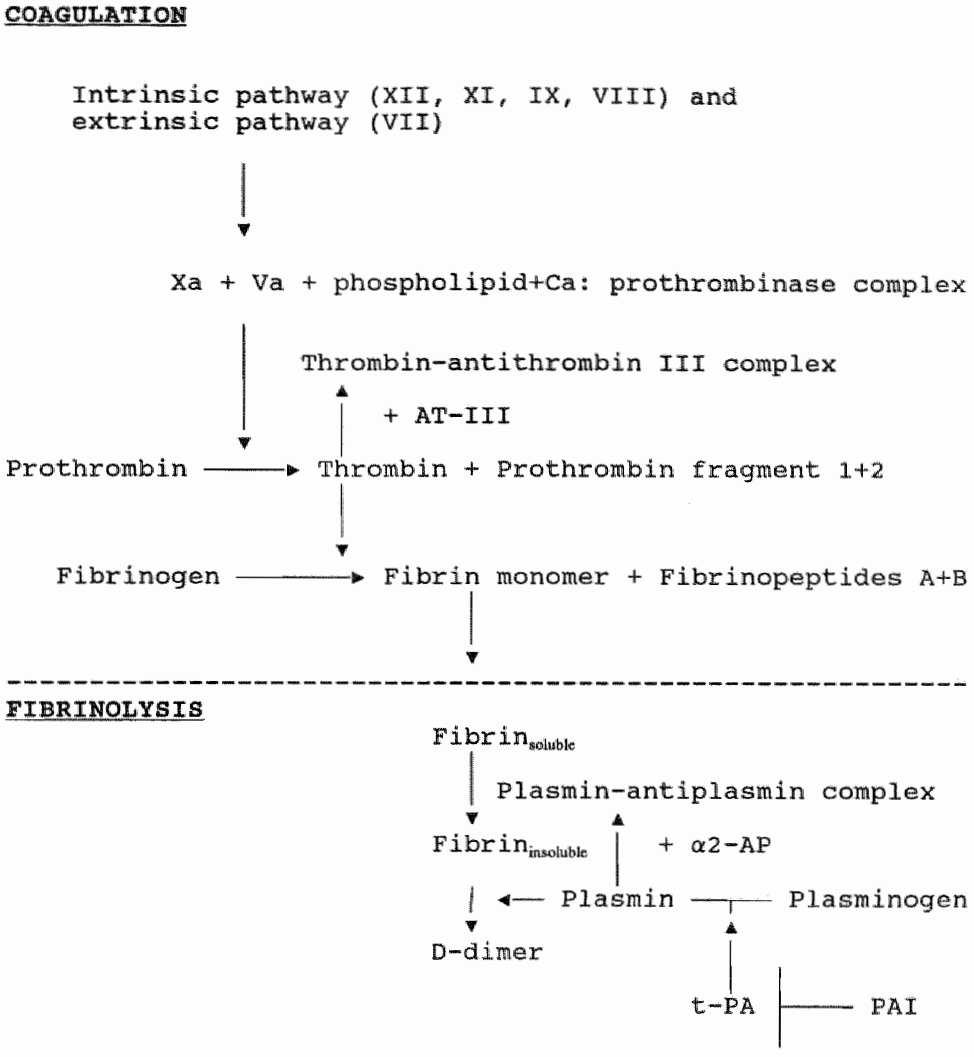

Fig 1. Coagulation and fibrinolysis

fibrinolysis in pregnancy, especially within the uteroplacental circulation. The plasmin-mediated degradation of cross-linked fibrin results in the formation of D-dimer fragments, which reflect activation of fibrinolysis.

Variables of haemostasis during pregnancy have been studied over the last two decades and they indicate that a normal pregnancy is accompanied by changes in the haemostatic system. This is thought to be a physiological adaptation necessary to ensure the integrity

of the expanding maternal and fetal circulation in the placenta and to control bleeding at the time of placental separation. Coagulation factors are increased during pregnancy, particularly in the last trimester. This is most striking in the case of plasma fibrinogen concentration[1-4], but is also observed with factors VII and VIII[5] and, to a moderate extent, with factors IX, X and XII.[6] Factors II and V remain unchanged whereas factors XI and XIII even show a decrease.[7-9] Antithrombin III (AT-III), an important inhibitor of thrombin, is unchanged in normal pregnancy[10,11], but is decreased in pre-eclampsia.[12]

Fibrinolytic activity has been reported as being reduced during pregnancy, particularly in the last trimester.[13-15] Stirling et al[5] described a marked decrease in fibrinolytic activity from 11-15 weeks of gestation. Most investigators have found that plasminogen levels increase during pregnancy[13-15] at the same time as the fibrinogen levels.[14] Levels of α_2-antiplasmin are also reported to increase.[15] Several lines of evidence suggest that smoking also affects the coagulation system as shown by higher plasma fibrinogen and TAT levels.[16] Decreased production of tissue plasminogen activator[17] and increased levels of plasminogen activator inhibitor[18] indicate a reduction of fibrinolysis in smokers. These effects are more pronounced immediately after smoking.[16]

In order to investigate the simultaneous effects of smoking and pregnancy on haemostasis a number of tests for parameters of the coagulation and fibrinolytic systems were performed. To monitor coagulation activation, levels of F 1+2, a sensitive marker for activation of coagulation[19] and TAT III, a marker of inhibition of coagulation[20], were measured. Plasminogen, α_2-antiplasmin and D-dimer levels were determined in order to measure the fibrinolytic activity. In combination, these tests yield information about changes in the balance between coagulation and fibrinolysis that may occur as a result of smoking during pregnancy. To our knowledge, studies in this field have not previously been reported.

Subjects and methods

The study was conducted in the de Wever Hospital, a teaching hospital serving a population of 200,000 inhabitants. Altogether, 101 pregnant women attending the department of obstetrics were included in the study. All participants gave their informed consent. The duration of the gestation was based on the last menstrual date and ultrasound determinations between 8 and 14 weeks. The number of cigarettes smoked per day was an estimate by the patient, in most cases confirmed by her partner. Non-smokers (n= 44) were defined as pregnant women reporting no smoking. All the smoking women (n= 57) consumed 20 cigarettes a day or more. Cotinine, a nicotine-specific metabolite and one of the most accurate biochemical assessments of nicotine exposure[21], was measured in the plasma of 20 (randomly selected) women to confirm their smoking habits. This was done in the second trimester of their pregnancy.

Exclusion criteria were a diastolic pressure ≥ 90 mmHg at the beginning or during pregnancy, an endocrine disease, a coagulation disorder or the use of medication known to interfere with the haemostatic system. Women with a history of (pre)eclampsia, hypertension, diabetes, a coagulation disorder, abruptio placentae, immature or premature delivery or a baby small for gestational age were excluded from the study. The characteristics of the women and their deliveries are summarized in Table 1. For the evaluation they were ranked into four groups according their gestational age: 0-10 weeks, 11-20 weeks, 21-30 weeks and 31-40 weeks of gestation. Not all patients were checked in all four stages.

Blood samples (altogether 201) were drawn between 8.30 and 9.00 a.m. after fasting overnight for ten hours followed by a resting period of 20 minutes. At least one hour had elapsed since the last cigarette. All parameters were determined in citrated plasma which was prepared by centrifugation of a mixture of 9 volumes freshly drawn blood with one volume trisodium citrate (0.11 mol/l) for 30 minutes (1600 g) at 25°C. The plasma was stored at -70°C in plastic tubes and thawed with tap water of 37°C for 5 minutes before serial analysis.

Prothrombin fragment 1+2 and thrombin-antithrombin III were assessed in plasma using an ELISA test of Behring Corporation (Marburg, Germany).

The D-dimer fibrin degradation products were measured by means of the ELISA test of Boehringer Mannheim, Germany. Tissue plasminogen activator antigen was also analysed using an ELISA technique (Innogenetics, Antwerp, Belgium). The same

Table 1. Basic characteristics of the pregnant women

Groups of patients	Age[a] (years)	Gestation[a] (days)	Birth weight[a] (g)	Placental weight[a] (g)	Blood loss [a]
Nulliparous					
Non-smokers (n= 20)	(28) (25-32)	40.1 (39.0-41.1)	3505 (3050-3890)	490 (410-610)	300 (250-500)
Smokers (n= 17)	29 (25-34)	39.0 (38.0-40.4)	2820 (2510-3310)	460 (400-540)	300 (200-400)
Parous					
Non-smokers (n= 24)	28 (25-32)	40.1 (39.0-41.1)	3600 (3178-3895)	510 (445-655)	300 (200-400)
Smokers (n= 40)	29 (25-34)	39.0 (38.0-40.4)	3060 (2665-3475)	480 (400-560)	300 (200-300)

[a] Values represent median (IR).

company supplied the plasminogen and α_2-antiplasmin reagents; Coatest plasminogen and Coatest α_2-antiplasmin, both using the chromogenic substrate S-2251.
The Mann-Whitney-Wilcoxon test was used for the statistical comparison of the median values.

Results

Table 2 shows the median values of prothrombin fragment 1+2 in the non-smokers and in the smokers. These fragments rose significantly in both groups from the beginning to the end of pregnancy; from 0.8 nmol/l to 2.5 nmol/l in the non-smokers group and from 1.0 nmol/l to 1.8 nmol/l in the smoking group. The smoking group showed significantly lower values in the second half of gestation (1.4 and 1.8 nmol/l versus 1.7 and 2.5 nmol/l in the non-smokers).
In the same Table the TAT concentrations in plasma of smoking and non-smoking pregnant women are compared. The concentrations rose significantly in both groups during pregnancy; from 2.2 µg/l to 9.9 µg/l in the non-smokers and from 3.1 µg/l to 8.5 µg/l in the smoking group. The median values in the non-smokers only differed significantly in the first ten weeks of gestation (2.2 µg/l versus 3.1 µg/l in the smoking group; $p= 0.016$).
The fibrinolysis variables in the smokers and in the non-smokers are illustrated in Table 3. This Table shows the median plasminogen levels in smokers and non-smokers during the four stages of gestation. There was a significant rise in plasminogen levels in the first half of pregnancy from 112% to 139% in the smoking group and from 103% to 140% in the non-smokers (resp. $p< 0.0001$ and $p= 0.001$), and a plateau was reached in the second half of gestation. The median α_2-antiplasmin levels are shown in the same table. These levels remained constant in both groups during pregnancy, but the smoking women started with significantly higher levels (119% versus 105%; $p= 0.009$). This Table also illustrates a progressive and significant increase in D-dimer fragments from the beginning to the end of pregnancy in the non-smokers (from 278 µg/ml to 847 µg/ml). This was also the case in the smokers, but only in the last three stages (from 278 µg/ml to 520 µg/ml). D-dimer levels in the smoking group were significantly lower in the last three stages of gestation in comparison to the values in the non-smoking group (stage IV 520 µg/ml in the smokers versus 847 µg/ml in the non-smokers; $p< 0.0001$).
Table 4 displays the median D-dimer/TAT III ratio in the two groups. The smoking women had significantly lower ratios at all four stages of pregnancy (stage I: 59 versus 117; $p= 0.006$: stage IV: 53 versus 101; $p= 5.5 \times 10^{-6}$).

Table 2. F1+2 and TAT III during pregnancy: comparison in plasma of smoking and non-smoking women at various stages of gestation[a]

	Gestational age (weeks)			
	0-10 NS (n=9) S (n=19)	11-20 NS (n=17) S (n=32)	21-30 NS (n=22) S (n=34)	31-40 NS (n=33) S (n=35)
	Median (25-75th percentile)	Median (25-75th percentile)	Median (25-75th percentile)	Median (25-75th percentile)
F 1+2 (nmol/l) Non-smokers	0.8 (0.6-0.9)	1.4 (1.2-1.7)	1.7 (1.4-1.9)	2.5 (1.9-3.0)
F 1+2 (nmol/l) Smokers	1.0 (0.8-1.3)	1.2 (0.9-1.5)	1.4 (1.2-1.7)	1.8 (1.6-1.9)
Significance S/NS p-value	n.s.	n.s.	0.03	0.00002
TAT (µg/l) Non-smokers	2.2 (1.8-2.8)	4.7 (3.9-6.0)	7.4 (6.6-9.5)	9.9 (7.5-12.0)
TAT (µg/l) Smokers	3.1 (2.8-5.7)	4.9 (4.1-6.6)	6.9 (5.4-8.6)	8.5 (7.1-11.9)
Significance S/NS p-value	0.016	n.s.	n.s.	n.s.

	Significance (p-value)		
	0-10 vs 11-20 weeks	11-20 vs 21-30 weeks	21-30 vs 31-40 weeks
F 1+2 (nmol/l) Non-smokers	n.s.	n.s.	0.0007
F 1+2 (nmol/l) Smokers	n.s.	0.027	0.001
TAT (µg/l) Non-smokers	<0.001	<0.001	0.019
TAT (µg/l) Smokers	0.042	0.003	0.003

[a] Abbreviations are defined in text; NS= non-smoking, S= smoking, n= sample size.

Table 3. Fibrinolytic variables[a]; values are shown as median (IR)

	Gestational age (weeks)			
	0-10 NS (n=9) S (n=19)	11-20 NS (n=17) S (n=32)	21-30 NS (n=22) S (n=34)	31-40 NS (n=33) S (n=35)
	Median (25-75th perc.)	Median (25-75th perc.)	Median (25-75th perc.)	Median (25-75th perc.)
Plasminogen (%) Non-smokers	103 (102-113)	140 (126-158)	147 (138-158)	145 (132-156)
Plasminogen (%) Smokers	112 (104-121)	139 (126-148)	151 (136-158)	151 (140-160)
Significance NS vs S	n.s.	n.s.	n.s.	n.s.
α2-antiplasmin (%) Non-smokers	105 (97-113)	107 (98-112)	107 (102-114)	102 (95-112)
α2-antiplasmin (%) Smokers	119 (117-129)	124 (119-133)	126 (120-131)	122 (115-128)
Significance NS vs S	0.009	<0.0001	<0.0001	<0.0001
D-dimer (µg/ml) Non-smokers	278 (210-504)	400 (303-567)	651 (420-847)	847 (681-1396)
D-dimer (µg/ml) Smokers	215 (165-255)	287 (220-360)	413 (290-470)	520 (400-620)
Significance NS vs S	n.s.	0.006	<0.0001	<0.0001

	Significance (p-value)		
	0-10 vs 11-20 weeks	11-20 vs 21-30 weeks	21-30 vs 31-40 weeks
Plasminogen (%) Non-smokers	<0.0001	n.s.	n.s.
Plasminogen (%) Smokers	0.001	n.s.	n.s.
α2-antiplasmin (%) Non-smokers	n.s.	n.s.	n.s.
α2-antiplasmin (%) Smokers	n.s.	n.s.	n.s.
D-dimer (µg/ml) Non-smokers	0.04	0.0001	0.003
D-dimer (µg/ml) Smokers	n.s.	0.04	0.02

[a] Abbreviations are defined in text, NS= non-smoking, S= smoking, n= sample size.

Table 4. Comparison of the D-dimer/TAT III ratios at different stages of gestation in plasma of smoking and non-smoking pregnant women

	Median D-Dimer/TAT III ratio (25-75 percentile)			
Gestational age (weeks)	0-10	11-20	21-30	31-40
Smokers	59[1)] (31-80)	56[2)] (35-71)	57[3)] (47-69)	53[4)] (42-71)
Non-smokers	117[1)] (74-158)	81[2)] (73-121)	84[3)] (55-124)	101[4)] (76-128)

[1)] vs [1)] p= 0.006; [2)] vs [2)] p= 0.001; [3)] vs [3)] p= 0.001; [4)] vs [4)] p= 5.5×10^{-6}.

Discussion

Both pregnancy and smoking affect the haemostatic system and render a subject "hypercoagulable". Coagulation parameters were found to be increased in smokers as well as in pregnant women.[1-9, 17-19, 22] Fibrinolytic activity has been reported to be diminished during pregnancy, particularly in the last trimester.[13-15, 22-24]
Studies of fibrinolysis in smokers have shown variable results. Two studies[19,25] reported that fibrinogen levels were higher and that fibrinolysis was decreased in subjects who smoked. In two other studies, fibrinolytic activity measured as euglobulin clot lysis was reported to be enhanced after smoking[26,27], whereas an other group of investigators found no influence of smoking on fibrinolysis[28]. Kimura et al.[29] studied the acute effect of cigarette smoking on haemostasis and found simultaneous increases in both coagulability and fibrinolysis during smoking.
It has been shown that an increase in the TAT concentration is a sensitive marker for activation of intravascular coagulation.[20,30,31] The present study shows that in healthy non-smoking, pregnant women activation of the coagulation system takes place, as demonstrated by the significant increase of TAT from the beginning to the end of pregnancy. This is in keeping with results from Reinthaller and co-workers[32] and an earlier study by our own group.[33] The TAT levels in the smoking group were not significantly different from those in the non-smoking group and they also showed a significant increase during pregnancy. A Japanese group found that TAT levels in peripheral venous blood increased significantly immediately after smoking[29] and remained elevated 41% above the baseline 15 minutes afterwards. These data suggest a transient activation of the coagulation system immediately after smoking. At this point one should be reminded that in the present study blood samples were not taken directly

after cigarette smoking, but at least one hour later. It seems that habitual smoking does not have an additive enhancing effect on the already activated coagulation in pregnancy. The fibrinolysis parameters showed a different pattern. In both groups the plasminogen levels increased significantly in the first 20 weeks of pregnancy to reach a plateau in the second half of gestation. Most investigators have found plasminogen levels to increase during pregnancy.[33,34] Bonnar[23] found that the increase in plasminogen levels occurred at the same time as the increase in fibrinogen, the proportional rate of increase in the third trimester being in the order of 50 to 60% with both fibrinogen and plasminogen. High plasminogen levels might be a protective measure against over-activation of clotting. Increased plasminogen levels could also be a reflection of diminished plasmin generation, indicating a decrease of fibrinolytic activity. Several authors found the α_2-antiplasmin activity to be increased during pregnancy[9,22] but in the present study the α_2-antiplasmin levels appeared to be constant. The levels of fibrin degradation products (D-dimer fragments) were found to rise progressively and significantly from the beginning to the end of pregnancy. These results are in accordance with a study from Stirling et al[5] and from our own group.[33] Evidence that the process of intravascular coagulation in normal pregnancy is confined to the placental area was produced by Van Royen[9] and later by Bonnar et al[35], who showed larger deviations from normal haemostasis in venous blood from the placental site than in blood from the forearm vein. The presence of raised FDP levels is therefore most likely to be the result of local (i.e. placental) degradation of fibrin.

In the present study, the smoking women showed the same plasminogen levels as the non-smoking group whereas their α_2-antiplasmin levels were significantly higher. The latter may imply a decrease in fibrinolytic activity, which is supported by the fact that D-dimer levels were significantly lower in the smoking group. The D-dimer/TAT ratio, which can be seen as an indicator of the balance between fibrinolysis and coagulation, was reduced in the smoking group compared to the ratio in the non-smokers. There is a lack of balance between fibrinolysis and coagulation activation in the smokers in the sense that the scale tips towards coagulation in them.

Conclusion

There is no indication of a reduction in fibrinolysis during normal pregnancy and the rise in D-dimer levels might even imply an increase. Habitual smoking does not have an additive enhancing effect on the already activated coagulation process in pregnancy. However, smoking during pregnancy leads to a reduction in fibrinolysis. In pregnant women who smoke the activated coagulation is not counterbalanced by an increase of fibrinolysis, as is the case in their non-smoking counterparts. This finding reflects an unfavourable effect of smoking during pregnancy.

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Chapter 9

Haematological variables in cord blood of neonates of smoking and non-smoking mothers

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Abstract

Smoking during pregnancy is associated with maternal and fetal complications. In the present study the effect of maternal smoking on neonatal cellular blood components was investigated. The values of whole blood cell count, leucocyte differential count, thrombocyte and reticulocyte count were determined and compared in cord blood of neonates of non-smoking (n= 89) and smoking (n= 53) mothers. The variables of the erythrocyte and thrombocyte count were not different in cord blood of neonates who were exposed to smoke and in those who were not. In the reticulocyte range the reticulocyte count was significantly lower in the smoking group, while the reticulocyte subsets remained stable. The neutrophils were significantly lower in cord blood of neonates of smoking mothers ($p < 0.05$). The latter finding might be an explanation for the enhanced incidence of postnatal infection seen in children of smoking mothers.

Smoking during pregnancy is associated with negative effects on the developing fetus such as increased frequency of pregnancy complications, reduced birth weight and increased perinatal mortality.[1,2] Immediate effects are decreased intervillous blood flow[3] and increased fetal heart rate.[4] Nicotine and carbon monoxide are the two main constituents in cigarette smoke exerting harmful effects on the fetus. Nicotine has a constricting effect on uterine blood vessels and interferes with the blood supply to the fetus. It has a direct vasoconstrictive effect on the fetus[5] and produces capillary damage in the placenta.[6] Carbon monoxide binds to haemoglobin and produces a conformational change in carboxyhaemoglobin that results in a shift of the oxygen dissociation curve leading to diminished transmission of oxygen to the tissues at a given oxygen tension, causing a mild form of hypoxia.[7]

Long term effects of smoking during pregnancy are also known. Children exposed to maternal smoking in utero are said to have significantly more neuropsychological deficits than children who have not been exposed.[8-10] An American epidemiological study showed an increased risk of 1.6 for malformations in children of mothers who smoked more than 20 cigarettes a day during pregnancy.[11] The available evidence is inconclusive on cancer risks for children of mothers who smoke during pregnancy. Pershagen et al[12] performed a cohort study, using information from the National Swedish Medical Birth and Cancer Registries. The maximum follow-up age was 5 years. They found no increase in overall cancer risk in children of mothers who reported smoking during pregnancy. Two studies indicated a clear dose related increase in cancer risk, which was particularly marked for acute leukemias.[13,14] The fetus can metabolize some of the genotoxic compounds found in tobacco smoke to DNA-binding metabolites. The presence of DNA adducts in fetal tissue is indicative of potential genomic damage that may result in an increased risk for the development of serious diseases, such as cancer in childhood or in later life.[15]

There is also a general agreement that exposure to environmental tobacco smoke also has an adverse effect on the health of children, especially on respiratory illnesses.[16] Since the early 1970s several studies reported an association between the smoking habits of parents and the pulmonary morbidity of their children.[16-18] Children with smoking parents have more respiratory infections, more respiratory problems such as asthma and, more hospital admissions for bronchitis and pneumonia.[19] It has been observed that childhood exposure to parental (mainly maternal) smoking is associated with the presence of asthma[20-24] and increased nonspecific bronchial hyperresponsiveness.[25] While studying the role of environmental tobacco smoke in the development of bronchial hyperresponsiveness in children Agudo et al[26] came to the conclusion that the only relevant source of environmental tobacco smoke exposure at home is the mother. The proximity between mother and child could explain this. A mean birth weight deficit of 88 g was found in newborns of non-smoking mothers whose fathers smoked more than 20 cigarettes a day.[27] Neonates born to mothers who smoke weigh about 200 g less than those born to non-smokers.[27] In conclusion, maternal smoking seems to be more important than paternal smoking in relation to the health of their offspring.

The effect of smoking on blood cells in adults and especially pregnant women is not yet clear. Cigarette smoking is known to be associated with elevations in the peripheral leucocyte count.[28] Little has been reported on the effect of chronic cigarette smoking on the erythrocytic system. It may decrease vitamin B_{12} [29] which could lead to an elevation of the mean corpuscular volume (MCV). Heavy long-term smoking may cause erythrocytosis.[30] The haemoglobin synthesis might be impaired by the lead content of tobacco.[31] Finally, smoking increases plasma viscosity.[32] An increase of platelet turnover and a decrease in platelet survival was found in smokers.[33,34] Platelet counts either remained normal[33,35] or were increased.[36,37]

Even less is known about the effect of smoking during pregnancy on blood cells in neonates. Nicotine and cotinine cause a reduction in uteroplacental blood flow and lead to hypoxia of the fetus[38] which in turn has been reported to provoke stimulation of the fetal erythropoiesis.[39-42] This is thought to increase the total red blood cell count, the haemoglobin and the haematocrit.[39,43] Reticulocytes are reported to increase.[39,40,44] Nevertheless, some authors claim that smoking has no relation to asphyxia[45] or to haemoglobin and haematocrit levels.[42,47] Neonatal blood platelets show little or no increase[48-50] or decrease.[39-51]

The mechanisms by which cigarette smoking produces adverse health effects are complex and multifactorial. The goal of the present study was to investigate whether these effects are reflected or mediated by haematological changes in the neonate. As changes in the leucocyte, erythrocyte, reticulocyte and thrombocyte range might be expected, the clinical relevance of haematological changes had to be assessed.

Subjects and methods

The study was done in the De Wever Hospital, Heerlen, The Netherlands, a teaching hospital serving a population of about 200,000. Hundred and ninety seven pregnant women, consecutively attending the department of Obstetrics between November 1992 and April 1993 were asked to participate in the study. Only five women refused. The duration of the gestation was based on the last menstrual date and ultrasound determinations between 8 and 14 weeks. The number of cigarettes smoked per day was an estimation by the patient, in most cases confirmed by her partner. Cotinine, a nicotine specific metabolite, was assessed in the plasma of the mother to confirm her smoking habits. This was done in the second trimester of their pregnancy. Cotinine is one of the most accurate biochemical assessments of nicotine exposure.[52] If a woman smoked more than four cigarettes a day she arbitrarily was categorized as a smoker. Non-smokers were defined as mothers reporting no smoking. There finally were 60 smokers and 110 women who did not smoke. Only 6 women smoked more than 15 cigarettes a day. This group was too small to subdivide the smokers into an intermediate smoking (5-15 cigarettes a day) and a heavy smoking group (> 15 cigarettes a day). Information on passive smoking (smoking partner) was not available.

Neonates whose mothers had a diastolic pressure ≥ 90 mm Hg, an endocrine disease or a coagulation disorder were excluded from the study, because these diseases might interfere with the haematological system. In the end the study group consisted of 142 singleton newborns, 64 males and 78 females, born from 89 non-smoking and 53 smoking mothers (Table 1). They were born at 34 weeks or more and with a birth weight between the 2.3th. and 97.7th. percentile for gestational age. Their Apgar score after 1 minute was 7 or more and the arterial umbilical cord pH was above 7.20. All neonates were declared healthy after examination.

Venous cord blood samples were taken of the clamped umbilical cord immediately after delivery. These samples were collected in EDTA-K_2 containing tubes (Sarstedt, Nümbrecht, Germany). To avoid in vitro changes of the blood cells they were kept at room temperature for a maximum of 3 hours before they were run on a Sysmex NE-8000 and a Sysmex R-3000 (Toa Medical Electronics Corp., Kobe, Japan).

The Sysmex NE-8000 is an automated haematology analyzer that uses the technology of radiofrequency and direct current measurement for cell counting and differentiation.[53] Reticulocyte counting was done by flow cytometry (Sysmex R-3000) which measures the absolute reticulocyte count and gives a cytogram, from which the reticulocytes can be subdivided into low (LFR), medium (MFR) and high (HFR) fluorescence ratio reticulocytes, in that order indicating the three stages in maturation of the reticulocytes.[54] Both instruments made it possible to obtain some relatively new haematological parameters that have clinical relevance. The earlier described reticulocyte subsets allow a more accurate evaluation of the bone marrow activity than does the total reticulocyte count.[55] The automated reticulocyte counter was evaluated for use in

Table 1. Basic characteristics of 89 non-smoking and 53 smoking pregnant women

Groups of patients	Age (years)[a]	Gestation (days)[a]	Frequency (%)	Birth weight[b] (g)	Placental weight[b] (g)
Nulliparous women					
Non-smokers	29.5 (27-32)	281 (275-287)	48.3	3395 (214)	517 (232)
Smokers	29.0 (26-31)	276 (273-283)	44.4	3116 (346)	487 (123)
Parous women					
Non-smokers	29.5 (27-32)	281 (275-287)	51.7	3605 (316)	521 (138)
Smokers	29.0 (26-31)	276 (273-283)	55.6	3309 (358)	544 (185)

[a] Values represent median (IR); [b] Values represent mean (SD).

paediatrics for investigating reticulocyte reference intervals as well as the corresponding maturity indices (LFR, MFR and HFR). Reticulocyte intervals showed no age difference for the period of 1 week to 16 years of age. Maturity grading revealed three data groups: the first 5 days of life, the first week to the end of the first month, and then up to 16 years of age.[56] Platelet size parameters, reflected by the mean platelet volume (MPV) and platelet distribution width (PDW), provide more information about platelet biology.[57-61] Platelets have been reported to deteriorate in functional ability with both decreasing size[57] and increasing age.[58]

The following list of constituents was determined:

1. In the erythrocyte range the total red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width-standard deviation (RDW-SD), red blood cell distribution width-coefficient of variation (RDW-CV).
2. In the reticulocyte range the total reticulocyte count (RET), low fluorescence ratio (LFR), mean fluorescence ratio (MFR) and the high fluorescence ratio (HFR).
3. In the thrombocyte range the total platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (which equals the percentage of cells with a volume of more than 12 fl) (P-LCR).

4. In the leucocyte range the total white blood cell count (WBC), neutrophilic granulocytes (NEUT), eosinophilic granulocytes (EO), basophilic granulocytes (BASO), monocytes (MONO) and lymphocytes (LYMPH).

The Mann-Whitney-U test was used to test the significance of differences between groups at the $p < 0.05$ level. A non-parametric test was used because the distribution of the values was not Gaussian.

Table 2. Comparison of the median and mean values of the erythrocyte variables in cord blood of newborns of non-smoking and smoking mothers[a]

Variable	Newborns of non-smokers (n=89)		Newborns of smokers (n=53)		Significance
	Median *Mean*	(25-75 perc) [X ± 2 SD]	Median *Mean*	(25-75 perc) [X ± 2 SD]	p-value
RBC (10^{12}/l)	4.40 *4.37*	(4.13-4.62) [3.60-5.20]	4.32 *4.32*	(4.15-4.68) [3.40-5.24]	n.s.
HGB (mmol/l)	10.0 *10.1*	(9.5-10.7) [8.1-11.9]	10.1 *10.1*	(9.6-10.6) [8.1-12.3]	n.s.
HCT (%)	47.9 *48.1*	(45.2-50.9) [38.2-57.2]	48.2 *48.2*	(45.1-50.9) [37.8-58.6]	n.s.
MCV (fl)	109.8 *110.1*	(107.4-113.3) [99.5-119.9]	109.8 *109.9*	(107.0-112.3) [98.5-121.1]	n.s.
MCH (fmol)	2.3 *2.3*	(2.2-2.4) [2.1-2.5]	2.3 *2.3*	(2.2-2.4) [2.0-3.1]	n.s.
MCHC (mmol/l)	21.0 *20.9*	(20.6-21.3) [20.6-22.5]	21.0 *21.1*	(20.7-21.5) [19.8-22.4]	n.s.
RDW-SD (fl)	67.7 *68.4*	(63.6-72.6) [51.6-83.6]	66.6 *67.8*	(63.0-72.1) [47.9-84.7]	n.s.
RDW-CV (%)	16.8 *17.1*	(16.1-17.7) [14.2-19.3]	16.5 *16.7*	(15.9-17.4) [13.5-19.5]	n.s.

[a] abbreviations are defined in text.

Results

In Table 2 the values of the erythrocyte variables in cord blood of newborns of smoking (n= 53) and non-smoking (n=89) mothers are shown. There were no significant differences between these two groups with regard to the erythrocyte range.
Table 3 shows that the median reticulocyte count was significantly lower in the newborns of smoking than of non-smoking mothers (149 x 10^9/l versus 170 x 10^9/l). The other reticulocyte variables were not different between the two groups.
Table 4 shows the median values of the thrombocyte range. These values were not different between the two groups.
In the leucocyte range the neutrophilic granulocytes were significantly lower in cord blood of children of smoking mothers (Table 5). In cord blood of non-smoking mothers the median value was 7.37 x 10^9/l compared to 6.01 x 10^9/l in cord blood of smoking mothers.

Table 3. Comparison of the median and mean values of the reticulocyte variables in cord blood of newborns of non-smoking and smoking mothers[a]

	Newborns of non-smokers (n=89)		Newborns of smokers (n=53)		Significance
	Median *Mean*	(25-75 perc) [X ± 2 SD]	Median *Mean*	(25-75 perc) [X ± 2 SD]	p-value
RET (10^9/l)	170.0 *166.0*	(145.8-192.6) [100.6-231.4]	149.0 *153.8*	(133.5-174.3) [75.7-231.9]	0.023
LFR (%)	61.5 *61.7*	(59.5-63.4) [54.5-68.8]	61.2 *61.5*	(58.8-63.8) [52.1-70.9]	n.s.
MFR (%)	24.2 *24.5*	(22.7-26.6) [18.4-30.5]	25.6 *25.2*	(23.3-27.2) [18.9-31.5]	n.s.
HFR (%)	14.0 *13.9*	(12.5-15.4) [8.3-19.4]	13.1 *13.3*	(11.6-15.4) [7.4-19.1]	n.s.

[a] abbreviations are defined in text.

Table 4. Comparison of the median and mean values of the thrombocyte variables in cord blood of newborns of non-smoking and smoking mothers[a]

	Newborns of non-smokers (n=89)		Newborns of smokers (n=53)		Significance
	Median *Mean*	(25-75 perc) [X ± 2 SD]	Median *Mean*	(25-75 perc) [X ± 2 SD]	p-value
PLT (10^9/l)	270 *269*	(237-321) [98-440]	277 *282*	(242-316) [131-433]	n.s.
PDW (%)	11.9 *11.9*	(11.1-12.5) [9.3-14.5]	11.9 *11.8*	(11.1-12.9) [9.4-14.2]	n.s.
MPV (fl)	10.2 *10.2*	(9.7-10.6) [8.8-11.6]	10.3 *10.3*	(10.1-10.9) [9.0-11.6]	n.s.
P-LCR (%)	26.1 *26.1*	(22.8-29.2) [15.0-37.2]	26.7 *26.7*	(24.3-30.7) [16.5-36.9]	n.s.

[a] abbreviations are defined in text.

Table 5. Comparison of the median and mean values of the leukocyte variables in cord blood of newborns of non-smoking and smoking mothers[a]

	Newborns of non-smokers (n=89)		Newborns of smokers (n=53)		Significance
	Median *Mean*	(25-75 perc) [X ± 2 SD]	Median *Mean*	(25-75 perc) [X ± 2 SD]	p-value
WBC (10^9/l)	13.3 *13.4*	(11.1-16.2) [5.2-21.5]	12.7 *13.0*	(9.8-15.3) [4.3-21.0]	n.s.
NEUT	7.4 *7.4*	(5.4-8.8) [1.7-13.1]	6.0 *6.2*	(4.0-7.1) [1.0-11.1]	0.028
LYMPH	3.8 *3.8*	(3.3-5.1) [1.2-6.5]	4.5 *4.4*	(3.0-5.1) [1.2-7.7]	n.s.
MONO	1.6 *1.6*	(1.2-2.3) [0-3.5]	1.8 *2.0*	(1.0-2.4) [0-4.2]	n.s.
EO	0.39 *0.4*	(0.23-0.54) [0-0.8]	0.35 *0.4*	(0.19-0.54) [0-0.8]	n.s.
BASO	0.06 *0.06*	(0.04-0.09) [0-0.10]	0.06 *0.06*	(0.04-0.08) [0.02-0.10]	n.s.

[a] abbreviations are defined in text.

Discussion

The effect of smoking on blood cells in adults and neonates is not yet clear. In adults cigarette smoking is known to be associated with elevations in the peripheral leucocyte count[28] and erythrocyte count.[30] Platelet counts either remain normal[33,35] or increase.[36,37] Information on the effect of smoking during pregnancy on neonatal blood cells is controversial.[38-51]

Prior to investigating the effects of maternal smoking on neonatal blood components, the effect of smoking during pregnancy on maternal haematological variables was studied.[62-65] Smoking in pregnancy appeared to have an additive enhancing effect on the already known leucocyte increase in pregnancy. The leucocyte differential count showed that the increase was mainly due to an increase of neutrophils, monocytes and lymphocytes.[62] The thrombocyte count did not differ in smoking and non-smoking pregnant women.[63] The erythrocyte count was significantly lower in smokers than in non-smokers. No statistically significant difference between the median haemoglobin and haematocrit levels was seen. The MCV was significantly higher in women who smoked, as was the MCH. The combination of the higher MCV and the lower erythrocyte count means less oxygen transport and might create a slight hypoxic condition for the fetus.[64] The absolute reticulocyte count was lower in the smoking group throughout pregnancy, but this was significant only in the last 10 weeks of gestation. There was no difference between the reticulocyte subsets of the smoking and the non-smoking group. Both groups behaved similarly during pregnancy: there was a decrease in the mature reticulocytes and an increase in the more immature reticulocytes.[65]

The analyses of the blood cells in the cord blood displayed quite another picture. No significant difference between the values of the total leucocyte count, the erythrocyte count and thrombocyte count was found in cord blood of the smoking and non-smoking group. In the leucocyte range only the neutrophilic granulocytes were significantly lower in cord blood of neonates of smoking mothers. The reticulocyte count was significantly lower in the smoking group, while the reticulocyte subsets, which allow a more accurate evaluation of the bone marrow activity than the total reticulocyte count[55], showed no differences between the two groups. One should be aware however that in view of the numerous variables tested the statistical differences might have occurred by chance.

In normal fetuses the lymphocyte count increases linearly with gestation. At 20 weeks the levels are about 50% of those at term.[66] These high numbers of lymphocytes are needed to acquire antigen recognition functions, necessary in case of viral infections that can pass the placenta. Neutrophil counts are low until 32 weeks and increase thereafter to adult levels at term ($1.8 - 8 \times 10^9/l$).[66]

In flow cytometric studies mature neutrophils are reported only at term.[67-69] This is supportive evidence for the hypothesis that the placenta forms an effective barrier for

most bacteria and that consequently a host-defense mechanism against bacterial infection is necessary only in the last trimester in preparation for extrauterine life.[70]
When a host acquires a bacterial infection, neutrophils are released from the neutrophil storage pool into the circulation and there is a compensatory increased proliferation of the mitotic neutrophils in the bone marrow.[71] Neonates have an increased susceptibility to infections, which has been attributed to immaturity of the phagocyte-macrophage system.[72,73] Neutrophils and monocytes play a crucial role in the host defense against pyogenic infections.[74-79] Harlap and Davies[17] found that the prevalence of hospital admission for infants with the diagnosis of bronchitis or pneumonia was twice as common in infants of smokers than in infants of non-smokers. Tager et al[80], using pulmonary function studies, documented decreased lung growth during childhood and adolescence in association with parental smoking. The effect on respiratory morbidity appears to be more, although not exclusively, related to maternal rather than paternal smoking. Bassi et al[81] developed a rat model of maternal smoking and demonstrated that fetuses suffered from growth retardation with a predominant effect on lung growth. Deficient formation of pulmonary septa suggests that growth of the connective tissue may have been impaired during pregnancy. It is not known whether the lung can recover from structural alterations due to antenatal maternal smoking. A study by Taylor and Wadsworth[18] supports the concept that maternal smoking influences the incidence of respiratory illnesses in children mainly through a congenital effect and only to a lesser extent through passive exposure after birth. No differences in the rates of hospital admission were found in children whose mothers started smoking only after delivery as compared with children of mothers who had never smoked. Neonates born from smoking mothers have significantly lower neutrophil counts than children born from non-smokers. This finding is congruent with the increased risk for postnatal infection in children of smoking mothers.
In summary, measurable effects of smoking on cord blood cell count were a reduction in the reticulocyte and the neutrophilic count. Thus, from a haematological point of view, the effect of smoking on the whole blood cell count of neonates is limited.

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Chapter 10

Cord blood cells and indices: smoking-related differences between the sexes

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Abstract

The values of whole blood cell count, leucocyte differential, platelet and reticulocyte counts were determined in cord blood of both male (n= 64) and female (n= 75) newborns of 87 non-smoking and 52 smoking mothers.
Leucocytes and neutrophils in cord blood from male newborns of smokers were significantly lower than those in their female counterparts and in the male newborns of non-smokers. These results suggest that male newborns are more affected by cigarette exposure than females with regard to some haematological parameters.

The effect of smoking during pregnancy on blood cells in neonates is not clear. Nicotine and cotinine cause a reduction in uteroplacental blood flow and lead to hypoxia of the fetus[1] which in turn provokes stimulation of fetal erythropoiesis.[2-5] In general, this is thought to increase the total red blood cell count, the haemoglobin and the haematocrit.[2,6] In addition, reticulocytes are reported to increase.[2,3,7] Nevertheless, some authors claim that smoking has no relation to asphyxia[8] or to haemoglobin or haematocrit levels.[9,10] The effect of smoking on neonatal blood platelets is also in dispute. Some authors describe little or no increase[11-13], but others suggest thrombocytopenia.[2,14] Since the late 1970s, the dangers associated with passive (involuntary) smoking have been widely debated. While research throughout the world has produced findings showing sidestream smoke to be harmful to non-smokers, an equal number of studies have indicated that the harm has been overstated or that it is non-existent.[15] There are as yet no data on the influence of passive smoking on blood cells in neonates.
In animal studies, nicotine has been shown to have sex-dependent effects.[16-18] Prenatal nicotine treatment reduced both the number of male pups and male birth weight but female pups tended to be unaffected. In human adults, males are also the more affected gender.[11,19] The aim of this study was to compare the erythrocyte, thrombocyte, leucocyte and reticulocyte counts in cord blood from female and male newborns of smoking and non-smoking mothers, in order to clarify the potential gender differences in haematological variables.

Subjects and methods

The erythrocyte, reticulocyte, thrombocyte, leucocyte and leucocyte differential counts were determined in venous umbilical cord blood from 139 singleton newborns (64 M, 75 F) born between November 1992 and April 1993 in the De Wever Hospital Heerlen, The Netherlands. All infants born at term (37-42 weeks' gestation) were included, irrespective of their birth weight. Their births were normal with Apgar scores of 7 or more after 1 min and arterial umbilical cord pH above 7.20. All neonates were declared healthy after examination. Their mothers were healthy pregnant women. Mothers with

a diastolic pressure ≥ 90 mmHg, an endocrine disease or a coagulation disorder were excluded from the study. The duration of gestation was based on the last menstrual date and ultrasound determinations between 8 and 14 weeks. The number of cigarettes smoked a day was estimated by the patient, and in most cases was confirmed by the partner. If a woman smoked more than 4 cigarettes a day she was defined as a smoker. Only six women smoked more than 15 cigarettes a day. This group was too small to subdivide the smokers into intermediate smoking (5-15 cigarettes a day) and heavy smoking groups (> 15 cigarettes a day). Non-smokers were defined as mothers reporting no smoking. Information on passive smoking (smoking partner) was not available.

Venous cord blood samples were obtained from the clamped umbilical cord immediately after delivery. These samples were collected in EDTA-K_2 containing tubes (Sarstedt, Nümbrecht, Germany) and kept at room temperature for a maximum of 3 h before being run on a Sysmex NE-8000 and a Sysmex R-3000 reticulocyte counter (Toa Medical Electronics Corp., Kobe, Japan). The latter instrument gives the reticulocyte count as a percentage of the erythrocytes, the absolute reticulocyte count, and a cytogram from which the reticulocytes can be subdivided into low (LFR), medium (MFR) and high (HFR) fluorescence ratio reticulocytes, in that order, indicating the three stages in the maturation of the reticulocyte. Another automated reticulocyte counter, the Sysmex R-1000 was evaluated for use in paediatrics for investigating reticulocyte reference intervals as well as the corresponding maturity indices (LFR, MFR, HFR). Reticulocyte reference intervals showed no age difference for the period 1 week to 16 years of age. Maturity grading revealed three data groups: the first five days of life, the first week to the end of the first month and then up to 16 years of age.[20]

The following list of constituents was determined: in the erythrocyte range, total red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width-standard deviation (RDW-SD), red blood cell distribution width-coefficient of variation (RDW-CV). In the reticulocyte range, the total reticulocyte count (RET) was determined, together with the proportions of LFR, MFR and HFR reticulocytes. In the thrombocyte range, total platelet count (PLT) was carried out, together with the platelet distribution width (PDW), mean platelet volume (MPV) and platelet large cell ratio (= percentage of cells with a volume more than 12 fl) (P-LCR). Counts of white cells (WBC), neutrophilic granulocytes (NEUT), eosinophilic granulocytes (EO), basophilic granulocytes (BASO), monocytes (MONO) and lymphocytes (LYMPH) were also performed.

The Mann-Whitney-U test was used to test the significance of differences between groups at the $p < 0.05$ level.

Results

In Table 1 the values of the erythrocyte, reticulocyte and leucocyte variables in cord blood of female newborns of smoking (n=29) and non-smoking (n=46) mothers are presented together with the results of male newborns of smoking (n=23) and non-smoking (n=41) mothers. Only significantly different values are shown. Of the erythrocyte variables, only RDW-CV was significantly lower in the total group of female newborns. The red cell count was 4.41 x 10^{12}/l in the male group compared with 4.34 x 10^{12}/l in the females. HGB was 10.1 mmol/l in the male group and 10.0 mmol/l in females. The reticulocyte count showed no significant differences in the two groups (151.5 x 10^9/l in the male group, 170.1 x 10^9/l in the females). The HFR reticulocytes were significantly fewer in the female group. Platelet count and platelet indices were similar in both groups: PLT 270 x 10^9/l in the males and 278 x 10^9/l in the females, PDW 11.9% in both groups, MPV 10.3 fl compared with 10.2 fl, P-LCR 26.6% versus 25.7%. The leucocyte range showed a different pattern. The male group had significantly lower leucocyte and neutrophil counts (12.0 x 10^9/l versus 14.3 x 10^9/l and neutrophil counts 5.9 x 10^9/l versus 7.4 x 10^9/l respectively). The lymphocyte, monocyte, eosinophil and basophil counts showed no differences.
These variables showed no significant differences in female and male newborns of non-smoking mothers. Male newborns of smoking mothers showed lower total leucocyte counts and neutrophil counts than female newborns of smoking mothers. The other

Table 1. Comparison of the median values of the erythrocyte, reticulocyte and leucocyte variables in cord blood of the total group of female newborns of non-smoking (n=46) and smoking (n=29) mothers and of male newborns of non-smoking (n=41) and smoking (n=23) mothers. Only significantly different values are shown

	Total group of male newborns	Total group of female newborns	Significance
	Median (25-75 perc.)	Median (25-75 perc.)	p-value
HFR (%)	14.7 (12.9-16.6)	13.0 (11.5-14.9)	0.014
RDW-CV (%)	17.2 (16.2-18.1)	16.4 (15.9-17.1)	0.008
WBC (10^9/l)	12.0 (10.4-15.2)	14.3 (12.4-16.8)	0.005
NEUT (10^9/l)	5.9 (4.5-8.0)	7.4 (5.6-8.9)	0.009

HFR = high fluorescence ratio, RDW-CV = red blood cell distribution width coefficient of variation, WBC = white blood cell, NEUT = neutrophilic granulocytes.

Table 2. Comparison of the median values of the leucocyte variables in cord blood of male newborns of non-smoking (n=41) and smoking (n=23) mothers. Only significant differences are shown

	Total group of male newborns of non-smokers	Total group of male newborns of smokers	Significance
	Median (25-75 perc.)	Median (25-75 perc.)	p-value
WBC (10^9/l)	12.9 (11.2-15.8)	10.7 (8.9-12.7)	0.013
NEUT (10^9/l)	6.5 (5.3-8.3)	5.1 (3.7-6.5)	0.018

WBC = white blood cell, NEUT = neutrophilic granulocytes.

variables were not significantly different. No differences were found when comparing these values in female newborns of smoking and non-smoking mothers. Comparison of the leucocyte counts in cord blood of male newborns of non-smoking (n= 41) and smoking (n = 23) mothers is shown in Table 2. The erythrocyte, reticulocyte, thrombocyte counts and indices in both groups were almost identical. In cord blood from male newborns of smoking women, total white cell counts and neutrophil counts were significantly lower compared with values in cord blood from male newborns of non-smokers. The median value for the white cells of male newborns of smoking mothers was 10.7 x 10^9/l compared with 12.9 x 10^9/l in male newborns of non-smokers. The median value for the neutrophil count in the group of newborns of smoking mothers was 6.5 x 10^9/l compared with 5.1 x 10^9/l in the group of newborns of non-smokers.

Discussion

Several animal studies have demonstrated that prenatal nicotine exposure affects male and female offspring differently. Peters and Tang[21] found that prenatal nicotine treatment of dogs reduced both the number of male pups born and male birth weight; females were not significantly affected. Riesenfeld[22] found that the body weight of male rats was reduced significantly more than that of female rats after prenatal nicotine exposure. Several studies investigating the effects of maternal smoking on fetal growth included sex-dependent effects. Ravenholt and Levinski[23] examined birth weight in 1,096 infants born to mothers who smoked regularly. There was a negative correlation between the number of cigarettes smoked and the proportion of males among live offspring. Wertelecki et al[18] analysed birth weight and length of 925 newborns whose mothers had smoked during pregnancy. Male full-term neonates whose mothers smoked

more than 10 cigarettes per day weighed 110 g less and were 1.1 cm shorter than those born to non-smoking mothers. The weight and length of the female newborns were not influenced by smoking.

In the present study the erythrocyte, reticulocyte and platelet ranges were not significantly influenced by smoking or gender and this was a significant finding. The white cell and neutrophil counts in cord blood were significantly decreased in male newborns of smoking mothers compared to those of non-smoking mothers and female newborns of smokers. Nicotine is known to have a direct toxic effect on neutrophils.[16]

The present study suggests that male newborns are more affected than females when haematological variables in cord blood of male and female newborns of smoking and non-smoking mothers are compared. The consequence of the decrease in the total white cell and the neutrophil counts might be a reduction in resistance to infection in the male newborn infant of the smoking mother[24,25] and this may persist throughout the neonatal period.[26] Neonates of smoking mothers tend to have higher rates of hospital admissions for bronchitis and pneumonia.[27] The present study suggests that male newborns of smoking mothers are more at risk of postnatal infections. Although more than 40 variables have been tested for statistical significance between the sexes and some differences might have occurred by chance, it seems desirable to increase the awareness of gender-specific sensitivity to cigarette smoke and to report such findings more systematically.

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Chapter 11

On haemostasis in newborns of smoking and non-smoking mothers

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Abstract

OBJECTIVE: To determine the effect of smoking during pregnancy on neonatal haemostasis.
STUDY DESIGN: Venous blood samples of 26 newborns of smoking and 25 newborns of non-smoking mothers were obtained from the clamped umbilical cord immediately after birth. Prothrombin fragment 1+2, thrombin-antithrombin III complex, plasmin-α_2-antiplasmin complex and D-dimer levels were determined to assess activation of coagulation and fibrinolysis. Cotinine was measured in all neonates to objectivate cigarette smoke exposure.
The Mann-Whitney-U test was used to compare the differences between the values of the parameters of coagulation and fibrinolysis in the two groups at the $p < 0.05$ level.
RESULTS: The median values of prothrombin fragment 1+2 and thrombin-anti-thrombin III complex in newborns exposed to tobacco smoke in utero did not differ from newborns who were not exposed. Nor were the markers of fibrinolysis, plasmin-α_2-antiplasmin complex and D-dimer levels, influenced by tobacco smoke exposure.
CONCLUSION: The balance between the components of coagulation and fibrinolytic pathways in neonates is not disturbed by maternal smoking.

Blood fluidity is maintained by the balance between the components of coagulation and fibrinolysis. After activation of the extrinsic and the intrinsic pathway prothrombin is formed, which converts to thrombin. Part of this thrombin forms a complex with antithrombin III (AT III), resulting in the coagulation activation marker thrombin-antithrombin III complex (TAT III). The remaining free thrombin leads to the formation of fibrin, which has to be degraded by fibrinolysis. For this purpose, plasminogen is converted into plasmin. Plasmin then degrades fibrin to D-dimer fragments, which reflects the activation of fibrinolysis (Fig. 1). This system is similar in newborns and adults, but the concentrations of several coagulation proteins differ in the newborn and are dependent on the gestational and postnatal age of the infant.[1-3] The generation of thrombin is decreased by approximately 50% in the newborn compared to the adult[4,5] and at the time of birth, AT III levels are approximately half adult values.[6] Most clotting factor levels in newborns are also lower than in adults.[7] The same is true for the fibrinolytic factors: the concentration of plasminogen is approximately 50% of the adult value.[7] On the other hand an important inhibitor of plasmin, α_2-antiplasmin, is relatively high with 85% of the adult value.[8] Up to now, the reason for the lower levels of these plasma proteins in neonates, as compared to the adult norm, is not clear and further studies are needed to elucidate the physiological background. Besides, the existing data on normal values for coagulation and fibrinolysis for newborns are incomplete and heterogeneous with regard to the quality of patient definition, specimen collection and testing methods. Moreover, the influence of smoking on the mentioned parameters has not been accounted for, although smoking can affect the haemostatic system. Cigarette smoking during pregnancy is associated with a well-documented

increase in perinatal mortality and morbidity rates.[9,10] Spinillo et al[11] found that maternal smoking in pregnancy increases the risk of intracranial haemorrhage in preterm infants.

The present study was performed with a double purpose. First, to see if usable reference values of parameters of coagulation and fibrinolysis for newborns were obtainable. Secondly, to investigate the influence of smoking during pregnancy on haemostasis in newborns. To monitor coagulation activation the levels of prothrombin fragment 1+2 (F 1+2)[12], and TAT III[13], were measured. Plasmin-α_2-antiplasmin complex (PAP) and D-dimer levels were determined in order to assess fibrinolysis activation.

Subjects and methods

Seventy-two pregnant women, consecutively attending the department of Obstetrics between April and June 1995 in the De Wever Hospital, Heerlen, The Netherlands were asked to participate in the study. None of the women refused. All women gave their informed consent. The duration of pregnancy was based on the last menstrual date and ultrasound determinations between 8 and 14 weeks. Exclusion criteria were a diastolic blood pressure ≥90 mmHg at the beginning or during pregnancy, an endocrine disease, a coagulation disorder, the use of medication known to interfere with the haemostatic system, or the history of any of these diseases, solutio placentae or immature or premature delivery. Twenty-one women had to be excluded for these reasons. All neonates were born at term (> 37 weeks) and were declared healthy after physical examination.

The number of cigarettes smoked per day by the mothers was an estimation by the patient, in most cases confirmed by her partner. Cotinine, a nicotine specific metabolite and one of the most accurate biochemical assessments of nicotine exposure[14], was measured in all neonates to objectivate cigarette smoke exposure. The last cigarette was smoked within 15 h before delivery. The cotinine measurements confirmed the women's smoking habits. In the end 26 newborns of smoking and 25 newborns of non-smoking mothers were included in the study. The basic characteristics of the mothers and their offspring are summarized in Table 1.

Venous blood samples of the newborns were obtained from the clamped umbilical cord immediately after birth. All parameters of haemostasis were determined in citrated plasma which was prepared by centrifugation of a mixture of 9 volumes freshly drawn blood with one volume trisodium citrate (0.11 mol/l) for 30 minutes (1600 g) at 25°C. The plasma was stored at -70°C in plastic tubes and thawed with tap water at 37°C for 5 minutes before serial analysis. F 1+2, TAT III and PAP were assessed in plasma using an Elisa test of Behring Corporation (Marburg, Germany). The D-dimer fibrin degradation products were measured by means of the Elisa test of Boehringer Mannheim, Germany.

Table 1. Basic characteristics of the mothers and of their newborns

Patients	Age[a] (years)	Gestation[a] (days)	Boy (B) Girl (G)	Delivery	Cigarettes[a]	Birthweight[a] (g)	pH[a]
Nulliparous women							
Non-smoking (n= 13)	28 (26-31)	276 (269-281)	5 B 8 G	F 1 V 2	0	3160 (2950-3650)	7.31 (7.27-7.32)
Smoking (n= 10)	26 (21-29)	281 (266-285)	6 B 4 G	V 2	10 (10-15)	2898 (2610-3540)	7.23 (7.18-7.30)
Parous women							
Non-smoking (n= 12)	31 (27-34)	273 (268-284)	6 B 6 G	V 1	0	3210 (2880-3610)	7.28 (7.23-7.33)
Smoking (n= 16)	30 (28-37)	270 (263-280)	8 B 8 G	V 1	13 (10-15)	3120 (2650-3510)	7.30 (7.26-7.33)

[a] Values represent median (IR); F= forceps delivery, V= vacuum extraction.

Table 2. Parameters of coagulation and fibrinolysis activation in newborns of smoking and non-smoking mothers[a]

	Newborns of smokers (n= 26)	Newborns of non-smokers (n= 25)	Significance	Laboratory reference range for newborns
	Median (25-75 perc)	Median (25-75 perc)	p-value	(2.5-97.5 perc)
F 1+2 (nmol/l)	1.5 (1.2 - 1.9)	1.7 (1.3 - 2.5)	n.s.	0.75 - 6.40
TAT-III (µg/l)	10.1 (5.4 - 14.0)	7.3 (6.0 - 25.0)	n.s.	1.7 - 75
PAP (µg/l)	190 (145 - 420)	360 (165 - 660)	n.s.	66 - 1250
D-dimer (µg/ml)	306 (243-582)	400 (291-922)	n.s.	177 - 1651
D-dimer/TAT III ratio	28.4 (16.1-41.4)	34.9 (25.6-49.1)	n.s.	2.5 - 68.9

[a] Abbreviations are defined in the text.

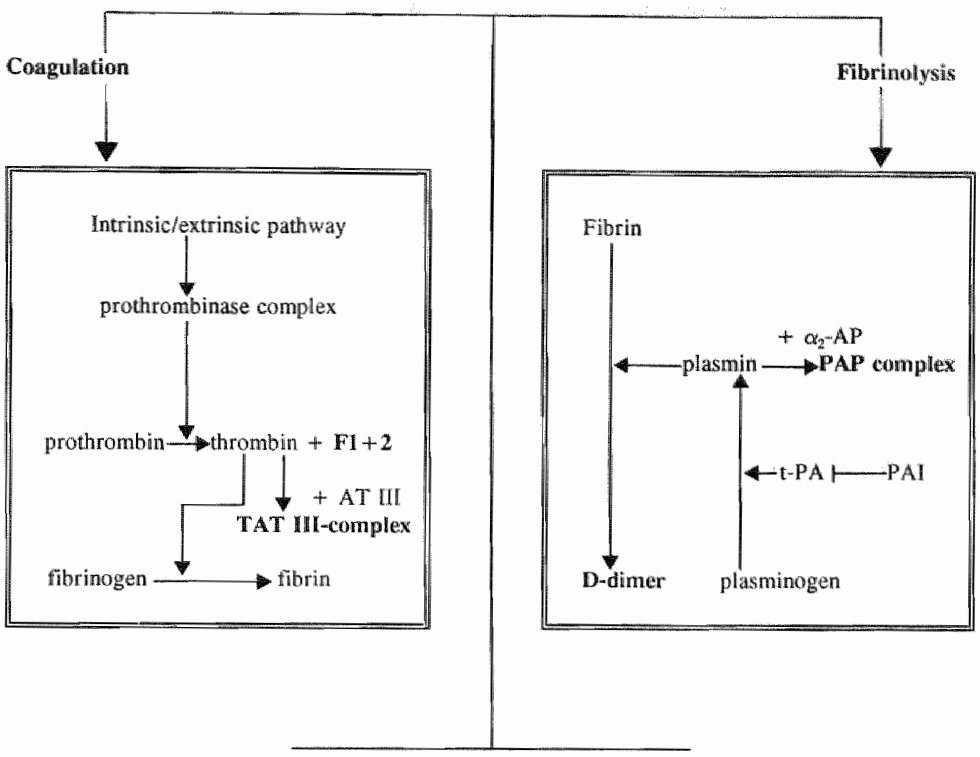

Fig 1. Homeostasis of the haemostatic system

Cotinine concentrations were measured by gaschromatography[15]. The detection limit for cotinine was 10 µg/ml.
The Mann-Whitney-U test was used to compare the differences between the values of the parameters of coagulation and fibrinolysis in the two groups at the $p< 0.05$ level.

Results

Table 2 shows the median values of F 1+2 and TAT III in newborns exposed to tobacco smoke in utero and in those who were not. Both parameters showed the same levels in the two groups. In the same Table the two markers of fibrinolysis are shown. PAP nor D-dimer levels were influenced by tobacco smoke exposure. While comparing each individual measurement of these four parameters it was found that all F 1+2, TAT, PAP and D-dimer values in the neonates of smokers were higher than the lowest values of these parameters in neonates of non-smokers. The D-dimer/TAT III ratio, which can

be seen as an indicator of the balance between fibrinolysis and coagulation, was the same in neonates of smokers and non-smokers (respectively 34.9 in the non-smokers and 28.4 in the smokers) (Table 2). The reference ranges (2.5-97.5 perc) of these parameters are given in the last column of Table 2.

Discussion

Knowledge of the normal development of the neonatal haemostatic system is necessary to interpret thrombotic and haemorrhagic episodes in the newborn. Coagulation factors are known to be changing constantly over the first months of life.[1,16] These changes are not only dependent upon the postnatal age of the infant but also upon the gestational age.[1,16] The prothrombin time (PT) and partial thromboplastin time (PTT) are often used to detect general abnormalities in the haemostatic system. Various reference values for newborns have been published for these tests.[17,18] Since normal values for the PTT and PT vary according to reagents used and there is no complete agreement on normal standards in adults, it is not possible to list age related normal values except as they relate to a single laboratory study.[19] Recently, assays for the measurement of activation products of the coagulation and fibrinolytic pathways have been developed.[20]

Conversion of prothrombin to thrombin, mediated by the prothrombinase complex (factor Xa, Va, calcium and phospholipid) is the key event in blood coagulation and produces a peptide designated F 1+2. The F 1+2 plasma level can be regarded as an indicator of the activity of the prothrombinase complex.[21] Reference values of F1+2 in newborns have not been published before.

Once formed in plasma, thrombin is inhibited by a naturally occurring antiproteinase, antithrombin III, with formation of a complex of TAT III.[22] AT-III levels in newborns are dependent on gestational age and birth weight.[23] The levels increase until term to about 50% of the adult norm.[24] Due to very low AT-III levels, newborns small for gestational age have an additional risk for thrombosis; since the AT-III pool of their plasma is small, they are prone to dysregulation in every situation of consumption.[24] Muntean et al[25] reported high TAT III levels shortly after birth in healthy term infants. He also observed that TAT III levels decreased to near normal adult values within 24 hours.

Plasmin activity can be assessed by measuring PAP.[26] There are no published reference data for PAP in neonates. D-dimers (D-fragments of fibrinogen and fibrin) are produced during plasmin mediated lysis of fibrin.[27] In adults the concentration of D-dimers is the most sensitive marker for monitoring the activity of intravascular coagulation.[28,29] Similar to TAT III, D-dimer levels were found to be high immediately after birth, returning to near normal values for adults within 24 hours after birth.[25,30]

Our findings in healthy term infants born after an uncomplicated pregnancy are consistent with a hypercoagulable state. TAT III levels were high immediately after birth.

The finding of the equally high D-dimer values indicate that fibrin formation is counterregulated by fibrinolytic activity. The D-dimer/TAT III ratio illustrates this. The large spreading of the reference values for these markers of coagulation and fibrinolysis makes them unusable for clinical practice.
Cigarette smoking during pregnancy is associated with an increase in perinatal morbidity and mortality.[9,10] In pregnant women who smoke the activated coagulation process is not counterbalanced by an increase of fibrinolysis, as is the case in non-smoking pregnant women.[31] Unlike their smoking mothers, neonates do not show a lack of balance between fibrinolysis and coagulation. In fact, comparing the four markers of haemostasis in newborns of smokers and non-smokers no differences were found.
In conclusion, the balance between the components of coagulation and fibrinolytic pathways in neonates is not disturbed by maternal smoking. A large population is now being studied to obtain usable reference values.

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Chapter 12

General discussion

The mechanisms by which cigarette smoking has a negative effect on pregnancy and pregnancy outcome are complex and multifactorial. The aim of this study has been to raise a corner of the veil by investigating whether these effects are mediated or reflected by changes in haematological variables in smoking pregnant women and/or their offspring.

During the course of this study the usual pitfalls in clinical research had to be confronted. First, smoking and non-smoking individuals live differently. It is known from studies on smoking and cardiovascular disease that smokers do not live as healthily as their non-smoking counterparts.[1,2] Secondly, this study was done in a large teaching hospital. The patients were seen by various doctors and were not all checked in the same four stages. Consequently, a careful interpretation should be made regarding the longitudinal changes in haematological parameters during the pregnancies of both the smoking and the non-smoking groups. A second study has now been initiated to provide more information on this subject.

Concentrations of nicotine and its metabolite cotinine were measured in the plasma of pregnant women to confirm their smoking habits. The results of these tests have shown that cotinine is more useful than nicotine in discriminating between non-smokers, light and heavy smokers. These results also showed that all the women gave truthful estimates of their smoking habits. Cotinine measurements were carried out in plasma of neonates of smokers and non-smokers and in neonatal plasma in parallel to maternal plasma. It became clear that cotinine is easily transferred to the fetal compartment. However, there seems to be a threshold of around 10 cigarettes per day. The latter finding could imply that the fetuses/ neonates of light smokers are not affected as far as cotinine is concerned. Nevertheless, the amounts transmitted of the other 3600 compounds in cigarette smoke are still unclear.

Some remarks should be made on the effect of smoking on blood cells and cell indices. Smoking in pregnancy leads to a lower erythrocyte count and a higher MCV. A lower erythrocyte count means less oxygen transport, whereas the transport of large erythrocytes is more difficult in constricted vessels. The production of PG I_2 in the umbilical cord is decreased by smoking, causing a constriction of the vessels in the placenta.[3] Because of a low blood pO_2 the fetus is hypoxic in utero in comparison to postnatal life. However, normally the fetus has access to the necessary amount of oxygen.

Two major adaptations make this possible. First, the haemoglobin of fetal red blood cells has a high affinity for oxygen. This property enables these cells to become highly saturated with oxygen as they circulate through the placenta. Secondly, the fetus has a high cardiac output in relation to its body size and metabolite rate. It is unlikely that this physiological state of hypoxia will be disturbed by maternal smoking. However, the effect of smoking could be of paramount importance during stressful episodes.

Smoking during pregnancy has an additive enhancing effect on the total leucocyte count, mainly due to an increase of neutrophils, monocytes and lymphocytes. This finding raises questions regarding the effects of smoking on immunity in pregnancy. Are lymphocyte subsets modified by smoking during pregnancy? And is humoral immunity an issue? Further research in this field might be rewarding.

The effect of smoking on the haemostatic system in pregnant women is interesting. The finding that the balance between coagulation and fibrinolysis is disturbed in this group can explain several obstetrical problems encountered in smokers, e.g. growth retardation, placental infarction and intrauterine death.

Smoking during pregnancy has a negative effect on the neutrophil count in newborns, especially male newborns. This might be an explanation for the enhanced incidence of bronchitis and pneumonia in children of smoking mothers. In adults, smoking can be considered as a chronic inflammatory disorder of the lower airways.[4] Tissue injury may result from the action of degenerative enzymes, from such cells as neutrophils and monocytes.[5] In addition, oxidative injury from cells and from oxidants in smoke may participate in the pathogenesis of smoking-related lung diseases.[6] Lung cancer represents the most striking risk imposed by cigarette smoking.[7] The cancer causing effects of cigarette smoking include the induction of carcinogen-activating enzymes and adduct formation with DNA, resulting in misreplication and mutation.[7] Smoking also exerts toxic effects that result in impaired mucociliary clearance, increased numbers of activated polymorphonuclear cells and macrophages, producing neutrophil elastase and other proteases, and decreased immunological responsiveness.[8] It remains to be investigated whether smoke exposure in utero predisposes the newborn to lung cancer in later life. A cohort study performed by Pershagen et al.[9] found no increase in overall cancer risk in children of mothers who smoked during their pregnancy. However, as the maximum follow up age was 5 years, further studies are needed to determine whether smoking during pregnancy induces malignancies in childhood or later life.

In the reticulocyte range the reticulocyte count itself was significantly lower in the smoking group whereas the reticulocyte subsets remained unchanged. The latter allow a more accurate evaluation of the bone marrow activity than the total reticulocyte count.[10] These results are remarkable as one would expect a stimulation of fetal erythropoiesis as a reaction to a lower erythrocyte count in maternal smokers. The variables of the erythrocyte and thrombocyte counts were not different in cord blood of neonates who were exposed to smoke from that of those who were not. Thus, from a

haematological point of view the effect of smoking on the whole blood cell count of neonates is limited.
Another remarkable finding is that neonatal haemostasis is not disturbed by maternal smoking. However, coagulation and fibrinolysis seem to be on a higher level in neonates of smokers. A larger population is now being studied.
The present study has brought to light some of the haematological effects of smoking during pregnancy. On account of these findings counselling of women who smoke should have a high priority in antenatal care. Pregnant women who continue to smoke should be counselled to stop for their own health and the health of their unborn child. Future research should focus on whether cigarette smoking or smoke exposure can change the functional characteristics of the blood cells. Molecular biological investigations will be necessary to obtain insight in the individual effects of the numerous components of cigarette smoke.

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Chapter 13

Summary

In the European community countries 36% of the inhabitants smoke. In the Netherlands 30% all women of reproductive age smoke, compared with 36% of men. Surveys of smoking during pregnancy have shown that the prevalence of smoking in this group of women is high. One-third of all pregnant women continue to smoke. Nicotine dependence is the most powerful driving force for continuing the habit.

In **chapter 1** the pharmacology of nicotine and its metabolite cotinine, carbon monoxide and thiocyanate is described. Cotinine measurements perform best in discriminating smokers and non-smokers because of its long half life (10-20 h) and its specificity for tobacco smoke exposure. Cotinine concentrations in the plasma and milk of the mother and the plasma and urine of the infant reflect the smoking habits of mothers during pregnancy.

Next, the effect of smoking on blood cells and haemostasis is reviewed. Various reports have established that total white blood cell counts are significantly higher in smokers compared to non-smokers. This raise has been attributed mainly to monocyte release. It remains difficult to resolve whether smoking increases or decreases inflammatory cell responsiveness. Carbon monoxide binds to haemoglobin, replaces oxygen and thus produces hypoxaemia, which in the long term can cause polycythaemia in smokers. Considerable evidence has accumulated to link habitual smoking and polycythaemia. Cigarette smoke has been reported to induce platelet activation, an effect mediated mainly by nicotine, and to increase the platelet adhesion to the vessel wall. Aggregation of platelets is acutely increased by cigarette smoke.

Maternal smoking during pregnancy may create a condition of chronic hypoxia for the fetus. This can be the result of the replacement of oxyhaemoglobin by carboxy haemoglobin. Additionally, structural changes in the placenta as well as decreased placental blood flow may also impair the oxygen supply to the fetus. Increased haemoglobin and haematocrit levels have been reported in infants of smoking mothers.

Several lines of evidence suggest that smoking affects coagulation status as shown by higher plasma fibrinogen and thrombin-antithrombin III levels. Studies of fibrinolysis in smokers have shown variable results. Both an increase and a decrease in fibrinolysis have been reported.

The effects of smoking on pregnancy are numerous as listed further. Cigarette smoking is associated with a dose-related reduction in fecundity and fertility. An increased risk

of spontaneous abortion and antepartum haemorrhage has been described. An average decrease in birth weight of 200 g has been reported. The association between smoking and perinatal death and maternal smoking and childhood cancer is disputable. Some tobacco related deaths are probably due to the increased risk of malformation. Children of mothers who smoke are admitted twice as often to the hospital for pulmonary problems.
The mechanisms by which cigarette smoking has a negative effect on pregnancy and pregnancy outcome are complex and multifactorial. The aim of this study was to investigate whether these effects are mediated or reflected by changes in haematological variables in smoking pregnant women and/or their offspring. Consequently, the following objectives were pursued:

1. The effect of smoking on blood cells and cell indices in mothers (chapter 4, 5, 6, and 7) and their newborns (chapter 9).
2. The sex-related differences of nicotine exposure in neonates (chapter 10).
3. The simultaneous effects of smoking and pregnancy on haemostasis (chapter 8).
4. The haemostasis in neonates of smoking and non-smoking mothers (chapter 11).
5. The estimation of the degree of fetal exposure to the constituents of cigarette smoke. For this purpose cotinine measurements were carried out in maternal blood plasma and cord blood plasma (chapter 3).

In **chapter 2** the literature regarding smoking and reproduction is reviewed. Cigarette smoking is associated in women with a dose-related reduction in fecundity and fertility and in men with a reduction of semen quality. Smoking has a negative effect on pregnancy: increased rates of antepartum bleeding and placenta praevia have been described. Smoking is also associated with increases in the rates of spontaneous abortion, low birth weight, perinatal death, and sudden infant death. Some tobacco-related perinatal deaths are due to an increased risk of serious malformation. Children of mothers who smoke are admitted twice as often to the hospital for pulmonary problems. Studies on maternal smoking and childhood cancer have proved inconclusive.
In **chapter 3** cotinine is measured in smoking pregnant women and their infants. Tobacco smoke consists of more than 3600 different compounds. One of its chief pharmacologically active ingredients is nicotine of which 60% is metabolized to cotinine. Cotinine is the best available biochemical measure of nicotine consumption because it is specific for tobacco smoke exposure and it has a long $t^1/_2$ (10-20h).
In the present study nicotine and cotinine measurements were carried out in 25 smoking and 25 non-smoking healthy pregnant women. In all 25 non-smoking pregnant women nicotine and cotinine levels were < 10 µg/ml. Light smokers (<10 cigarettes/day) were found to have nicotine blood levels < 10 µg/ml and cotinine levels varying between 40 and 99 µg/ml. Heavy smokers (≥10 cigarettes/day) had nicotine levels < 10 µg/ml, but high cotinine levels varying from 115 to 199 µg/ml. Cotinine measurements were carried out in 25 neonates of non-smoking mothers and in 34 neonates of smoking mothers.

The mothers of 9 of these 34 newborns were included in the study to investigate the relationship between maternal and neonatal cotinine concentrations. Cotinine levels in neonates of non-smokers and women who smoked less than 10 cigarettes/day were below the detection limit of 10 µg/ml. Cotinine values in neonates whose mothers smoked ≥ 10 cigarettes/day were significantly higher than in those whose mothers smoked ≤ 10 cigarettes/day, but significantly lower than in their mothers. There seems to be a threshold of around 10 cigarettes/day.

Cotinine measurements in the pregnant women confirm that cotinine measurements are more useful than nicotine in discriminating non-smokers, light and heavy smokers. Cotinine concentrations were significantly lower in the neonates than in their mothers, but there was a strong positive linear relationship between maternal and neonatal cotinine concentrations.

In **chapter 4** the leucocyte count is studied in 194 smoking and 518 non-smoking healthy pregnant women. Smoking in pregnancy appeared to have an additive enhancing effect on the already known total leucocyte increase in pregnancy. The leucocyte differential count in 105 smoking and 288 non-smoking pregnant women showed that the eosinophil and basophil count was not involved in the white blood cell shift. The rise of the total leucocyte count was mainly due to an increase of neutrophils, monocytes and lymphocytes. The leucocytosis in the smoking pregnant women was dose-related: significant upward jumps of the percentages of leucocytosis were observed between 12 and 15 cigarettes/day as well as between 19 and 20 cigarettes/day.

In **chapter 5** the erythrocyte count and indices are compared in 247 non-smoking and 123 smoking healthy pregnant women at four different stages of pregnancy: 0-10, 11-20, 21-30 and 31-40 weeks. Blood samples were run on a Sysmex NE-8000. The erythrocyte count was significantly lower in smokers than in non-smokers (3.86×10^{12}/l versus 3.96×10^{12}/l) in the last ten weeks. Comparing the erythrocyte count at the beginning and the end of pregnancy there were significant lower values in both groups (4.32×10^{12}/l to 3.96×10^{12}/l in the non-smoking and 4.24×10^{12}/l to 3.86×10^{12}/l in the smoking group). The differences in the median HGB and HCT levels were neglectable. The MCV was significantly higher in women who smoked, as was the MCH (MCV 91 fl and MCH 1.90 fmol in the non-smoking versus MCV 94 fl and MCH 1.95 fmol in the smoking group) in the last ten weeks. Smoking in pregnancy leads to a lower erythrocyte count and a higher MCV which might create a hypoxic condition of the fetus.

In **chapter 6** the reticulocyte count and its subfractions are compared in 247 non-smoking and 123 smoking healthy pregnant women at different stages of normal pregnancy. Blood samples were run on the Sysmex R-3000 reticulocyte counter. This instrument is able to provide precise reticulocyte counts. Furthermore, it estimates the maturity of reticulocytes by measuring the fluorescence intensity, a reflection of the RNA content of the cell. As the reticulocytes become older, their fluorescence (RNA content) decreases. The high fluorescence ratio is therefore a reflection of the most immature

reticulocyte. The absolute reticulocyte count showed a trend to lower values in the smoking group throughout pregnancy, but this was only significant in the last ten weeks of gestation (71.9 x 10^9/l versus 78.8 x 10^9/l). There was no difference between the low fluorescence, the medium fluorescence and the high fluorescence proportions in the non-smoking and the smoking group. Both groups behaved similarly during pregnancy; there was a decrease of mature reticulocytes and a significant increase of more immature reticulocytes. These data show a moderate measurable effect of cigarette smoking on the reticulocyte count and the absence of an effect on the reticulocyte subsets.

In **chapter 7** the platelet count and indices are compared in the same population. Most platelet studies have been confined to platelet counts in normal pregnancy, although the combination of platelet and platelet size parameters as mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) might provide better insight in platelet biology during pregnancy. Blood samples were run on the Sysmex NE-8000. There was no significant difference between the platelet count in the two groups. In the non-smoking group, the platelet count showed a significant decrease with gestational age (287 x 10^9/l to 258 x 10^9/l). This was not the case in the smokers group. The MPV of the smokers was significantly lower than that of the non-smokers in the last ten weeks of pregnancy (10.4 fl versus 10.7 fl). The PDW and the PCT did not change under the influence of cigarette smoking. The present study indicates that smoking during pregnancy does not affect platelet count and platelet indices in a clinically relevant way.

In **chapter 8** coagulation and fibrinolysis in smoking (n= 57) and non-smoking (n= 44) pregnant women are discussed. In order to investigate the simultaneous effects of smoking and pregnancy on haemostasis prothrombin fragment 1+2 (F 1+2) and thrombin-antithrombin III (TAT III) (parameters of coagulation activation) and plasminogen, α_2-antiplasmin and D-dimer levels (parameters of fibrinolysis) were assessed. There was no indication of a reduction of fibrinolysis during normal pregnancy. Habitual smoking does not have an additive enhancing effect on the already activated coagulation process in pregnancy. However, smoking during pregnancy did lead to a reduction of fibrinolysis. In pregnant women who smoke the activated coagulation was not counterbalanced by an increase of fibrinolysis, as was the case in their non-smoking counterparts.

Chapter 9 deals with the effect of maternal smoking on neonatal cellular blood components. The values of whole blood cell count, leucocyte differential count, thrombocyte, erythrocyte and reticulocyte count were determined in cord blood of neonates of non-smoking (n= 89) and smoking (n= 53) mothers. The variables of the erythrocyte and thrombocyte count were not different in cord blood of neonates who were exposed to smoke and in those who were not. In the reticulocyte range the reticulocyte count was significantly lower in the smoking group, while the reticulocyte subsets remained stable. The neutrophils were significantly lower in cord blood of neonates of smoking mothers. The latter finding might be an explanation for the enhanced incidence of postnatal infections seen in children of smoking mothers.

In **chapter 10** the same parameters as in chapter 9 were assessed in cord blood of both male (n= 64) and female (n= 75) newborns of 87 non-smoking and 52 smoking mothers. Leucocytes and neutrophils in cord blood from male newborns of smokers were significantly lower than those in their female counterparts and in the male newborns of non-smokers. These data suggest that male newborns are more at risk of postnatal infections. Although more than 40 variables have been tested for statistical significance between the sexes and some differences might have occurred by chance, it seems desirable to increase the awareness of gender-specific sensitivity to cigarette smoke and to report such findings more systematically.
In **chapter 11** the effect of maternal smoking on neonatal haemostasis has been studied. Twenty-six newborns of smoking mothers and 25 newborns of non-smoking mothers were included. To monitor coagulation activation, levels of prothrombin fragment 1+2 (F 1+2) and thrombin antithrombin III (TAT III) were measured. Plasmin-α_2-antiplasmin complex (PAP) and D-dimer levels were determined in order to assess fibrinolytic activity. The findings in healthy term infants born after an uncomplicated pregnancy were consistent with a hypercoagulable state. TAT III levels were high immediately after birth. The finding of the equally high D-dimer values reflects coagulation activation with reactive fibrinolysis in the newborn. This physiological process was not influenced by tobacco smoke exposure.
In **chapter 12** the previous chapters and future research goals are discussed. Future studies should focus on the question whether cigarette smoking changes the functional characteristics of blood cells. Investigations in the field of molecular biology are necessary to obtain insight in the individual effects of the numerous compounds of cigarette smoke.

In summary, it can be concluded that:

1. Cotinine, the principal metabolite of nicotine, is easily transferred to the neonatal compartment. There seems to be a threshold of around 10 cigarettes/day.
2. Smoking during pregnancy has an additive enhancing effect on the total leucocyte count.
3. Smoking in pregnancy leads to a lower erythrocyte count and a higher mean cell volume (MCV).
4. Smoking during pregnancy does not affect reticulocyte and platelet count and indices in a clinically relevant way.
5. In pregnant women who smoke the activated coagulation process is not counterbalanced by an increase of fibrinolysis, as is the case in their non-smoking counterparts.
6. Smoking during pregnancy has a negative effect on the neutrophil count in neonates. Male newborns are more affected by cigarette exposure than female newborns.
7. The balance between the components of the coagulation and fibrinolytic pathways in the neonates is not disturbed by maternal smoking.

8. On account of these findings counselling of women who smoke should have a high priority in antenatal care. Pregnant women who continue to smoke should be counselled to stop for their own health and the health of their unborn child.
9. Future studies should focus on the effect of smoking on the functional characteristics of blood cells. Studies in the field of molecular biology will be necessary to obtain more insight in the individual effects of the numerous components of tobacco smoke.

Chapter 14

Samenvatting

In West-Europa rookt 36% van de bevolking. Van de Nederlandse vrouwen in de vruchtbare levensfase rookt 30%. Voor mannen in dezelfde leeftijdscategorie bedraagt dit percentage 36. Uit onderzoek naar rookgedrag blijkt dat een derde van de Nederlandse vrouwen tijdens de zwangerschap blijft roken. Afhankelijkheid van nicotine is de belangrijkste oorzaak van dit gedrag.

In **hoofdstuk 1** wordt de farmacologie van nicotine, cotinine, koolmonoxide en thiocyanate beschreven. Het bepalen van de cotinine-concentratie is de beste methode om onderscheid te maken tussen rokers en niet-rokers. De cotinine-concentraties in plasma en melk van de moeder en in plasma en urine van het kind zijn immers goede graadmeters voor het objectiveren van het rookgedrag tijdens de zwangerschap.

Vervolgens wordt een overzicht gegeven van het effect van roken op de bloedcellen en de haemostase. In meerdere onderzoeken is aangetoond dat bij rokers het totale aantal witte bloedcellen groter is dan bij niet-rokers. Deze toename betreft met name het aantal monocyten. Het blijkt moeilijk vast te stellen of roken de reactie van de ontstekingscellen verhoogt of verlaagt. Koolmonoxide bindt zich aan haemoglobine, hetgeen kan leiden tot hypoxaemie en op termijn tot polycythaemie. Het verband tussen roken en polycythaemie is vaker beschreven. Roken leidt tot activatie van de thrombocyten. Dit effect wordt met name aan nicotine toegeschreven en leidt tot een verhoogde adhaesie van de thrombocyten aan de vaatwand. De aggregatie van deze cellen wordt acuut versterkt door roken.

Het roken tijdens de zwangerschap kan leiden tot een chronische hypoxie bij het kind. Enerzijds kan dit effect het gevolg zijn van de vervanging van oxyhaemoglobine door carboxyhaemoglobine. Anderzijds kan het roken leiden tot structurele veranderingen in de placenta en een verminderde placentaire doorbloeding, hetgeen een verminderde zuurstofvoorziening voor de foetus betekent.

Roken heeft ook invloed op de coagulatie. Onderzoeken laten bij rokers hogere waarden zien voor fibrinogeen en thrombine-antithrombine III. Het effect van roken op de fibrinolyse is nog onduidelijk, omdat zowel toename als afname van fibrinolyse worden gemeld.

In hoofdstuk 2 wordt een uitgebreid overzicht gegeven van de uit de literatuur naar voren komende mogelijke gevolgen van het roken door vrouwen tijdens de vruchtbare levensfase en de zwangerschap.

De mechanismen, die leiden tot de negatieve effecten van het roken tijdens de zwangerschap zijn zeer complex. In de volgende hoofdstukken wordt onderzocht wat de betekenis is van haematologische veranderingen bij de rokende moeder en/of haar kind voor de verklaring van die mechanismen. Het onderzoek richt zich daartoe met name op:

1. Het effect van roken op bloedcellen en indices bij moeders (de hoofdstukken 4, 5, 6 en 7) en pasgeborenen (hoofdstuk 9).
2. De sexe-afhankelijke verschillen in de gevolgen voor pasgeborenen van het rookgedrag van de moeder tijdens de zwangerschap (hoofdstuk 10).
3. De simultane effecten van roken en zwangerschap op de haemostase (hoofdstuk 8).
4. De verschillen in haemostase tussen pasgeborenen van rokende en van niet-rokende moeders (hoofdstuk 11).
5. Het bepalen van de cotinine-concentratie in maternaal en navelstreng-bloed om vast te stellen in welke mate de foetus bij een rokende moeder wordt blootgesteld aan rookcomponenten (hoofdstuk 3).

In **hoofdstuk 2** wordt een overzicht gegeven van de literatuur met betrekking tot roken en reproductie. Roken wordt geassocieerd met een dosisafhankelijke afname van bevruchting en vruchtbaarheid bij vrouwen en met een verminderde spermakwaliteit bij mannen. Roken heeft ook een negatief effect op het verloop van de zwangerschap. Er wordt een toename van bloedingen en placenta praevia beschreven. Roken wordt verder in verband gebracht met miskramen, laag geboortegewicht, perinatale sterfte en wiegedood. De perinatale sterfte is in een aantal gevallen te wijten aan een verhoogde kans op ernstige malformaties. Kinderen van rokende moeders worden tweemaal zo vaak in het ziekenhuis opgenomen vanwege longproblematiek. De relatie tussen roken tijdens de zwangerschap en het bij het kind optreden van kanker op jeugdige leeftijd is nog onzeker.
In **hoofdstuk 3** worden de resultaten weergegeven van de meting van cotinine-concentraties bij rokende moeders en hun kinderen. Tabaksrook bestaat uit meer dan 3600 verschillende stoffen. Nicotine is een van de farmacologisch meest actieve componenten. Nicotine wordt omgezet in cotinine en die stof vormt de beste biochemische maatstaf voor objectivering van rookgedrag. Cotinine is namelijk specifiek voor het inademen van tabaksrook en het heeft een lange halfwaardetijd (10-20 uur). Nicotine- en cotininemetingen werden verricht bij 25 rokende en 25 niet-rokende gezonde zwangere vrouwen. Bij alle niet-rokende vrouwen werden nicotine- en cotininewaarden < 10µg/ml gevonden. Bij vrouwen die minder dan 10 sigaretten per dag rookten was de nicotinewaarde < 10µg/ml en varieerde de cotininewaarde tussen 40 en 99 µg/ml. Bij een gebruik van 10 of meer sigaretten per dag bleef de nicotinewaarde laag (< 10 µg/ml), maar steeg de cotininewaarde naar 115 tot 199 µg/ml.
Bij 25 pasgeborenen van niet-rokende moeders en 34 pasgeborenen van rokende moeders werden de cotinine-concentraties bepaald. De moeders van 9 van deze 34 neonaten namen deel aan het onderzoek om de relatie tussen maternale en neonatale cotininewaarden te bestuderen. De cotininewaarden bij pasgeborenen van niet-rokers

en lichte rokers waren ≤ 10 µg/ml. De cotininewaarden bij pasgeborenen van moeders, die 10 of meer sigaretten per dag rookten, waren significant hoger dan bij pasgeborenen van moeders die minder rookten, maar significant lager dan de waarden bij hun eigen moeders. Voor de cotinine-overdracht van moeder naar kind lijkt er sprake te zijn van een drempel als de consumptie beneden de 10 sigaretten per dag blijft.
Metingen bij zwangere vrouwen bevestigen dat via cotininebepaling een beter zicht op het werkelijk rookgedrag verkregen kan worden dan via nicotinebepaling.
De cotinine-concentraties bij neonaten zijn significant lager dan bij hun moeders, maar er is een sterke positieve lineaire relatie tussen de maternale en de neonatale cotinine-concentraties.
In **hoofdstuk 4** worden de resultaten van leucocytentelling bij 194 rokende en 518 niet-rokende gezonde zwangere vrouwen beschreven. Roken doet het tijdens de zwangerschap toch al stijgend aantal leucocyten nog verder toenemen. De leucocytendifferentiatie bij 105 rokende en 288 niet-rokende zwangere vrouwen toont aan dat de eosinofielen en de basofielen niet en de neutrofielen, monocyten en lymfocyten wel aan deze toename bijdragen. Deze leucocytose bij rooksters is dosisafhankelijk. Er wordt een significante toename van het percentage leucocytose gezien bij de toename van het aantal sigaretten per dag van 12 naar 15 en van 19 naar 20.
In **hoofdstuk 5** worden de erythrocyten, erythrocytenindices alsmede de haemoglobine- en haematocrietwaarden bij 247 niet-rokende en 123 rokende zwangere vrouwen vergeleken. De metingen werden verricht in de volgende stadia van de zwangerschap: 0-10, 11-20, 21-30 en 31-40 weken. De bepalingen werden uitgevoerd met behulp van de Sysmex NE-8000. Het aantal erythrocyten bleek in de laatste 10 weken van de zwangerschap bij rooksters significant lager dan bij niet-rooksters: 3,86 x 10^{12}/l versus 3,96 x 10^{12}/l. In alle gevallen was er sprake van een daling van het aantal erythrocyten. Van 4,32 x 10^{12}/l tot 3,96 x 10^{12}/l bij de niet-rooksters en van 4,24 x 10^{12}/l tot 3,86 x 10^{12}/l bij de rooksters. De verschillen in de mediane haemoglobine- en haematocrietwaarden waren verwaarloosbaar klein. De MCV was, evenals de MCH, in de laatste 10 weken van de zwangerschap significant hoger bij de rooksters: MCV 91 fl en MCH 1,90 fmol bij de rooksters en 94 fl en 1,95 fmol bij de niet-rooksters.
Roken tijdens de zwangerschap leidt tot een lager aantal erythrocyten en een hogere MCV, hetgeen onder bepaalde omstandigheden kan leiden tot een hypoxie bij de foetus.
In **hoofdstuk 6** worden de reticulocyten en de subfracties daarvan bij rooksters en niet-rooksters vergeleken. De benodigde bepalingen werden uitgevoerd met behulp van de Sysmex R-3000 reticulocytenteller. Dit apparaat verstrekt exacte aantallen reticulocyten en geeft informatie over de rijpheid van deze cellen door bepaling van de fluorescentie-intensiteit als weerspiegeling van de RNA-inhoud van de cel. De RNA-inhoud van reticulocyten vermindert met het ouder worden. Een hoge fluorescentieratio duidt derhalve op veel jonge reticulocyten.
Het aantal reticulocyten was lager bij rooksters, maar dit was alleen in de laatste 10 weken significant: 71,9 x 10^{9}/l versus 78,8 x 10^{9}/l. Er was geen verschil in de fluorescentieratio's

tussen rooksters en niet-rooksters, terwijl er bij beide groepen sprake was van een afname van het aantal onrijpe reticulocyten.
In **hoofdstuk 7** worden de thrombocyten-aantallen en -indices bij rooksters en niet-rooksters vergeleken. Vroeger uitgevoerde onderzoeken beperkten zich meestal tot het tellen van de thrombocyten tijdens de zwangerschap. Bij het onderhavige onderzoek werd echter ook de mogelijkheid betrokken dat het combineren van thrombocytenaantal met indices zoals gemiddeld plaatjesvolume (MPV), de distributiewijdte van plaatjes (PDW) en plateletcrit (PCT) een beter inzicht in het effect van roken op de biologie van de trombocyten zou kunnen geven.
Bij de niet-rooksters daalde het aantal thrombocyten significant tijdens de zwangerschap: van 287 x 10^9/l naar 258 x 10^9/l. Bij de rooksters was dit niet het geval. De MPV was gedurende de laatste 10 weken van de zwangerschap bij rooksters significant lager dan bij niet-rooksters: 10,4 fl versus 10,7 fl. De PDW en PDT veranderden niet onder invloed van roken.
In **hoofdstuk 8** worden coagulatie en fibrinolyse bij rokende (n=57) en niet-rokende (n=44) zwangere vrouwen onderzocht. Prothrombine fragment 1+2 (F1+2) en thrombine-antithrombine III (TAT III) werden bepaald als graadmeters voor de stollingsactivatie; plasminogeen, α_2-antiplasmine en D-dimeren als graadmeters voor de fibrinolyse.
Roken bleek geen additioneel effect te hebben op de tijdens zwangerschap toch al geactiveerde coagulatie. Roken leidde wel tot een afname van de fibrinolyse. Bij rokende zwangere vrouwen wordt de geactiveerde coagulatie derhalve niet gecompenseerd door een toename van de fibrinolyse, hetgeen wel het geval is bij niet-rooksters.
In **hoofdstuk 9** worden gevolgen van het roken door de moeder voor de bloedcellen van de pasgeborene onderzocht. Daartoe werden leucocyten, thrombocyten, erythrocyten, reticulocyten en de indices daarvan bepaald in navelstrengbloed van 89 pasgeborenen van niet-rokende moeders en 53 pasgeborenen van rooksters. Aantallen en indices van erythrocyten en thrombocyten leverden geen verschillen op tussen beide groepen. Het aantal reticulocyten was significant lager bij kinderen van rooksters, maar de reticulocytensubsets waren niet verschillend. Het aantal neutrofielen was eveneens significant lager bij de kinderen van rokende moeders. Deze bevinding zou een verklaring kunnen vormen voor het feit dat de kinderen van rokende moeders vaker luchtweginfecties hebben.
In **hoofdstuk 10** wordt aandacht besteed aan mogelijke sexe-afhankelijke gevoeligheid voor tabaksrook. Daartoe werden de in hoofdstuk 9 genoemde waarden bepaald in navelstrengbloed van 64 mannelijke en 75 vrouwelijke pasgeborenen van 87 niet-rokende en 52 rokende moeders. Aantallen leucocyten en neutrofielen waren significant lager bij mannelijke dan bij vrouwelijke pasgeborenen van rooksters.
Ondanks het feit dat het onderzoek zich op meer dan 40 variabelen richtte, en sommige verschillen mogelijk aan toeval te wijten zijn, lijkt het wenselijk aandacht te besteden aan sexe-afhankelijke gevoeligheid voor tabaksrook.

In **hoofdstuk 11** wordt het effect van roken op de neonatale haemostase beschreven. Bij dit onderdeel van het onderzoek waren 26 pasgeborenen van rokende en 25 van niet-rokende moeders betrokken. De coagulatie werd onderzocht door middel van bepaling van F 1+2 en TAT III en de fibrinolyse door middel van plasmine-α_2-antiplasmine-complex (PAP) en D-dimeerbepalingen. Hoge TAT III- en D-dimeer-concentraties in de gezonde pasgeborenen duiden op coagulatie activatie met reactieve fibrinolyse. Dit fysiologische proces blijkt bij pasgeborenen niet beïnvloed te worden door het rookgedrag van de moeder.
In **hoofdstuk 12** worden de voorafgaande hoofdstukken en mogelijke toekomstige onderzoeksprojecten besproken. In die projecten zou met name aandacht besteed kunnen worden aan de vraag of roken de functie van bloedcellen verandert. Moleculairbiologisch onderzoek zal nodig zijn om meer inzicht te verkrijgen in de effecten van de talrijke afzonderlijke bestanddelen van tabaksrook.

Samenvattend kunnen de volgende conclusies getrokken worden:

1. Cotinine, de belangrijkste metaboliet van nicotine, wordt gemakkelijk getransporteerd naar de foetus en de neonaat. Dat lijkt met name het geval wanneer de moeder meer dan 10 sigaretten per dag rookt.
2. Zwangerschap veroorzaakt een stijging van het aantal leucocyten. Roken tijdens de zwangerschap leidt tot een verdere toename van het aantal leucocyten.
3. Roken tijdens de zwangerschap veroorzaakt een daling van het aantal erythrocyten en een toename van het gemiddelde celvolume (MCV).
4. Roken tijdens de zwangerschap heeft geen klinisch relevant effect op het aantal reticulocyten en de subfracties daarvan.
5. Bij zwangere vrouwen die roken, wordt de geactiveerde coagulatie niet gecompenseerd door een toename van de fibrinolyse. Bij niet-rooksters is dat wel het geval.
6. Roken tijdens de zwangerschap veroorzaakt een daling van het aantal neutrofielen bij de pasgeborene. Bij mannelijke pasgeborenen is dit in hogere mate het geval dan bij vrouwelijke.
7. Bij pasgeborenen blijkt het evenwicht tussen coagulatie en fibrinolyse niet verstoord als hun moeders tijdens de zwangerschap rookten.
8. Op basis van bovenstaande conclusies dient vrouwen in de vruchtbare levensfase te worden geadviseerd om te stoppen met roken. Dit zowel terwille van haar eigen gezondheid, als terwille van de gezondheid van haar ongeboren kind.
9. Het lijkt zinvol voortgezet onderzoek met name te richten op het effect van roken op de functie van de bloedcellen. Moleculair biologisch onderzoek zal nodig zijn om meer inzicht te verkrijgen in de effecten van de talrijke afzonderlijke bestanddelen van tabaksrook.

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Het bloedonderzoek vond plaats binnen de afdeling Haematologie van hetzelfde ziekenhuis (hoofd: Dr. J. van Wersch).

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Luc, bo ta mi premio major!

Curriculum vitae

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